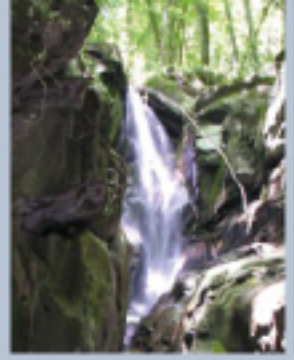
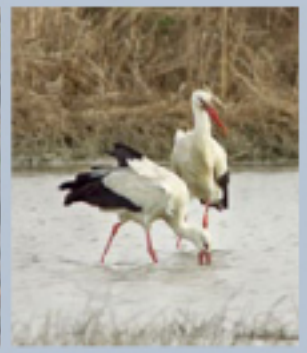
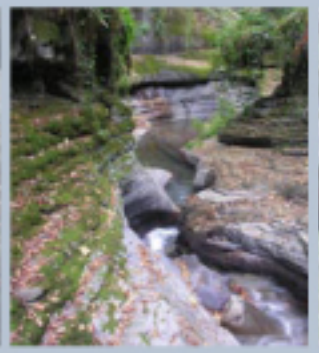


With the contribution of the LIFE financial instrument of the European Commission

Following the water course





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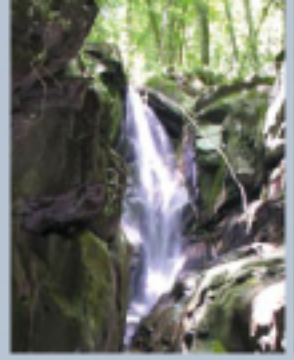

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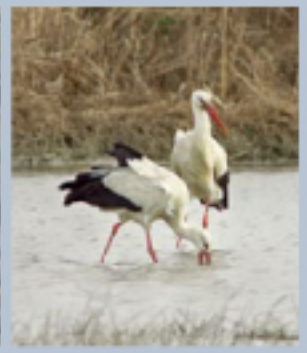

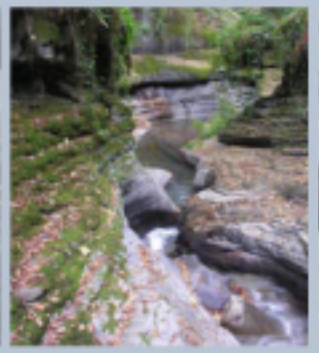


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Nothing in the world is softer and weaker than water,
yet nothing is better at overcoming the hard and strong.
(Lao-Tze, Tao Te Ching)

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PREMISE

The "Water Scis" LIFE project: an example of the efficient use of community funding

The Province of Prato is known for being home to one of Europe's most developed and structured textiles districts, constructed out of the rubble of the second world war, and which has succeeded in creating and distributing work and wellbeing throughout all of the social strata in the resident population. However, this undeniable success story has had the "collateral effect" of partially obscuring other potentials which the territory has to offer – in particular, the area's significant wealth of cultural heritage and the variety and size of its natural heritage. Given that residential and industrial development has focussed mainly on the urban areas of the plains close to the River Bisenzio, the more outlying areas of the plains and the foothills of the Apennines still retain some areas of considerable natural value, which are often "mixed" with the cultural heritage.

The "Water SCIs" LIFE community project, led by the Province of Prato in partnership with the Lakes Suviana and Brasimone Natural Park, had the merit and the ambition of aiming to requalify these fragments of territory still in a good state of conservation, despite the numerous threats surrounding them. The ultimate goal of the project was (and remains) to improve the conservation status of the Species of Community Interest (SCI) that are not adequately represented or are exposed to risks in the upper Apennine area and the plain surrounding Prato. The aquatic environment is essential for important phases in these species' life cycles, and it is precisely the relationship with this element that explains the enigmatic name chosen for the project: "Water SCIs".

The successful strategy chosen to achieve the conservation goals was to act on several parallel levels at the same time:

- knowledge: through appropriate scientific means, we obtained as reliable and complete a picture as possible of the presence, distribution and conservation status of the target species and any competitor species before, during and after the project;
- regulatory: in order to ensure adequate protection for the environments and species chosen, we succeeded in extending the Special Protection Area (SPA) *Ponds of the Florentine and Prato Plain* in order to include the wetland areas affected by the environmental requalification measures, as well as establishing the Site of Community Interest (SCI) *Appennino Pratese* in order to protect the watercourses and important habitats present, as well as approving the SPA Management Plan and specific conservation action plans for *Cottus gobio* (a small-sized benthic fish) and *Austropotamobius pallipes* (the white-clawed crayfish);
- operational: thanks to community funding, we performed a range of concrete conservation measures, such as salvaging wetland areas, building a fish nursery, creating fish ladders, implementing experimental measures to encourage *in-situ* and *ex-situ* breeding of *C. gobio* and *A. pallipes*, and experimenting methods of controlling invasive alien species;
- participation: throughout the project duration, we remained constantly committed to seeking possible forms of collaboration with all those interested in the project, both through institutional opportunities to participate in the procedures approved by the competent authorities and through specific initiatives to raise awareness of the protected environments, the contents and goals of the community project and of the LIFE programme and the European "Natura2000" network of conservation sites in general.

As well as achieving all our goals, despite the difficult economic period and the institutional overhaul going on during the period of implementation, the project succeeded in raising awareness among citizens of the high value of the elements of natural heritage still present in the area in which they live. This recognition contributed to increasing the resident population's awareness and sensitivity to environmental issues, which is the only solid guarantee of long-term protection for these important habitats and the species they host and will continue to welcome in the future, to the benefit of current and future generations.

Stefano Arrighini

Councillor for the Development of Natural Resources and
Protected Areas in the Province of Prato

PREMISE

Protecting biodiversity in the current institutional framework

Running a structured and complex EU funded project like the one described in this publication required the beneficiaries (the Province of Prato and the Lakes Suviana and Brasimone Natural Park) to deal with some important challenges, capable of improving the project's capacity and extending the professional skills of the staff and structures who drafted the proposal and subsequently conducted the project.
The "Water SCIs" LIFE project is not the first time the Province of Prato has been involved in a similar project; in the past the Province also acted as coordinator of the LIFE "HABIO: Biodiversity protection in the Calvana-Monferrato areas" project in 2001-2004.
The reason for this ongoing commitment to protecting biodiversity lies, at least partly, in the specific structure and skills that the current administrative framework (both in terms of its own and delegated functions) grants to Provinces, including: protecting flora and fauna, parks and nature reserves; land protection; safeguarding and valorising the environment; protecting and valorising water resources; hunting and fishing in inland waters; rural development. Provinces are also responsible for drafting and implementing territorial coordination plans outlining the general goals and guidelines for the area.

As is clear from reading these pages, the fact that these functions are performed by a single institutional figure allows the Province to take a leading role in all decision-making regarding environmental issues in general. Moreover, these powers are not only exercised by the Province during the regulatory phase of the project (by issuing permits, authorisations, grants, approval of large-scale territorial planning, sector planning, etc.), but also during the control phase (through the Provincial police force).
This institutional framework, which is currently being completely overhauled, effectively grants Provinces the full responsibility for biodiversity (confirmed by the relevant Regional regulations, Regional Law 56/00), in keeping with the administrative principles of subsidiarity, differentiation and adequacy set out in Article 118 of the Italian Constitution. In fact, the principle of subsidiarity means that institutional figures responsible for larger areas, such as Provinces, must intervene when figures responsible for smaller areas, such as Municipalities, do not have adequate resources to achieve specific targets or effectively protect specific interests. This is supported by the principle of adequacy, which requires the institutional figure responsible for a certain function to equip itself with the necessary resources to effectively perform its duty.

These principles have been fully implemented in the "Water SCIs" LIFE project, allowing the Provincial Administration to:

- Submit a proposal to the Tuscan Regional Authority to set up a Site of Community Interest and extend a Special Protection Area;
- Approve the Management Plan for the Special Protection Area;
- Approve the conservation Action Plans for the two species of community interest;
- Mitigate the possible impact of plans and projects for Natura2000 sites by authorising measures to improve and/or compensate for activities through an Environmental Impact Assessment;
- Include specific requirements in Hunting Regulations to maintain adequate water levels in the wetlands and maintain plantlife;
- Regulate the competitor fish species introduced in the watercourses subject to specific protection measures due to the presence of species or habitats of conservation interest;

- Introduce regulations in the Technical Implementation Rules for the Territorial Coordination Plan to protect species and habitats of interest, whose distribution has been recorded and mapped during specific surveys conducted by Research Centres and Institutes;
- Ensure compliance with these regulations, with the aid of the Provincial police force.

As a result, it is clear how the Provincial Administration, in its current form, along with Regional and National Parks, are the most suitable institutional figures to implement community projects aiming to protect biodiversity and the Natura2000 sites, as the only figures capable of guaranteeing, through territorial and sectorial planning and with the aid of the Provincial police force, both the maintenance of the conservation measures introduced thanks to community funding and the limitation of factors that could threaten their effect, for a time period extending well beyond the necessarily limited duration of the projects approved.

The current phase of institutional overhaul must take these aspects into account, and above all the need to identify a figure specifically responsible for conservation management, to ensure that protecting biodiversity does not simply remain a "good intention", let down by strategic development choices and ultimately absent from the reality of territorial transformation.

Arch. Carla Chiodini

Director of the Territorial Planning and
Land Protection Department at the Province of Prato

INTRODUCTION

THE VALUE OF BIODIVERSITY

Biodiversity is a relatively new concept, but one that has quickly established itself, not only in the scientific field, indicating "the variability among living organisms of any origin, including, among others, terrestrial, marine and other aquatic ecosystems and the ecological complexes to which they belong; this includes diversity within species and between species and diversity of the ecosystems" (Article 2 of the United Nations Convention on Biodiversity).

This definition refers to three levels of diversity: 1) within a single species (for example, the countless "variations on a theme" shown by breeds, varieties or ecotypes belonging to the same species, obtained as a result of natural or artificial selection); 2) between species (whether they are animals, plants or micro-organisms); 3) between ecosystems (woods, grasslands, bodies of water, etc.). However, there is also a fourth, no less important, level consisting of functional biodiversity – i.e. the range of interactions within and between the three levels described. It is precisely these interactions that guarantee the survival of living species: in fact, in order to adapt to the changing environmental conditions, species require to suitable and diversified habitats and ample genetic variability, which represents the "field of activity" in which natural selection occurs. At the same time, in order to work properly, ecosystems rely on the whole variety of species that they host.

In this sense, protecting terrestrial and marine biodiversity represents a kind of "life insurance for the planet", making it possible for the quantity and quality of the goods and services that nature offers to all species, including our own, to be maintained over time.

Humanity (despite our lack of awareness) benefits from an enormous wealth provided free of charge by planet Earth, called **natural capital:** a patrimony of essential goods and services such as food, textile fibres, drinkable water, breathable air, carbon dioxide capture and climate stabilization, to mention just a few.

In the period between 2001 and 2005, the United Nations commissioned a group of 1,360 experts from all over the world to draft an international report called the Millennium Ecosystem Assessment[1] which, among other things, introduced the following classification of ecosystem services:

Ecosystem **support** services: for example, the nutrient cycle, soil formation, photosynthesis, etc.

Ecosystem **supply** services: for example, the availability of food, fresh water, wood and fibres, fuel, genetic resources, etc.

Ecosystem **regulating** services: for example, climate regulation, flood regulation, disease regulation, water purification, erosion regulation, pollination, etc.

Ecosystem **cultural** services: for example, aesthetic, spiritual, educational and recreational value, etc.

This is unmistakeable evidence that the well-being of our species depends entirely on the continuous flow of these "ecosystem goods and services" over time. These public goods are nearly all free, without a market or prices; as a result, they are not given due consideration by the currently dominant economic system, which is eroding the natural capital in an alarming manner that could ultimately threaten the long-term maintenance of conditions suitable for life on our limited planet.

To put it in a way that is immediately comprehensible, this economic model or paradigm fails to assign adequate value to an asset that is not only useful, but also essential, like water, while it attaches great value to goods, such as jewellery, that certainly aren't particularly useful in terms of the survival of a species or an ecosystem. These real "errors of judgement", caused by applying strict market principles to our natural capital, are proving to be among the main underlying causes of the degradation of ecosystems and the loss of biodiversity that we are witnessing.

Among the pioneering scientific works that have investigated the relationship between classical economics and natural resources, some particularly interesting ones include Westman's article published in "Science" back in 1977[2], the 1997 text edited by G.C. Daily[3] and the one written by the "founding father" of ecology as a scientific discipline, Eugene Odum[4]. There is also a specific branch of research dedicated to the topic, known as "Ecological-economics", to which the names of top profile researchers such as Herman Daly, Robert Costanza, H.T. Odum, Kenneth Boulding, David Pimentel, Nicholas Georgescu-Roegen (theorist of so-called "bio-economy"), K. William Kapp and Karl Polanyi are linked.
These topics are becoming increasingly relevant, especially in the current phase of prolonged social and economic crisis which is prompting us to rethink our social and economic models, and are the focus of a global initiative called "The Economics of Ecosystems and Biodiversity" (TEEB)[5] which has taken on the ambitious challenge of *making the values of nature visible*, i.e. helping policy-makers to recognize, demonstrate and define the value of ecosystems and of biodiversity and to give due consideration of these values as part of the decision-making process.

Protecting of biodiversity on an international, European, national and regional level

The Convention on Biological Diversity (CBD) was implemented in Nairobi (Kenya) on 22 May 1992, with the participation of 193 of the planet's 204 nations. The Convention opened with the signature of these countries during the World Summit in Rio de Janeiro in June 1992 (Earth summit) in conjunction with the United Nations Framework Convention on climate change and the Convention to Combat Desertification.
The primary objectives of the CBD were: 1) the conservation of biological diversity, 2) the sustainable use of its components, and 3) the fair and equitable sharing of the benefits arising from the use of genetic resources, through proper access to genetic resources and appropriate technology transfer, taking into account all rights over these resources and technologies, and through adequate funding (Art. 1 of the Convention).
After declaring 2010 the International Year of Biodiversity, the United Nations defined the period 2011-2020 as the "UN Decade for Biodiversity", to contribute to the implementation of the **Strategic Plan for Biodiversity**[6], divided into seven thematic programmes, corresponding to the planet's major biomes:
1) rural environments; 2) dry and sub-humid areas; 3) forests; 4) inland waters; 5) islands; 6) sea and coasts; 7) mountain areas.

2011-2020 has also been set aside within the EU as the decade for implementing the **European Biodiversity Strategy**[7] (Communication COM/244 of May 2011), with the following key objective for 2020: to put an end to the loss of biodiversity and the degradation of ecosystem services in the EU by 2020 and restore them, as far as possible, while at the same time intensifying the EU contribution to preventing the loss of biodiversity worldwide. This key objective consists of and is broken down into in the following six priority objectives:

1. Promote the implementation of environmental legislation;
2. Reinstate the ecosystems, for example by using green infrastructures;
3. Stimulate sustainable agriculture and forestry;
4. Encourage sustainable fishing;
5. Tackle invasive alien species;
6. Contribute to stopping the loss of biodiversity at a global level.

The European Commission implements its policy of nature conservation and biodiversity by setting up an ecological network known as "Natura 2000", funded by a financial instrument called the "LIFE Programme".

Natura 2000 is a coherent ecological network of protected sites (currently called pSCI – proposed Site of Community Interest) spread throughout the entire territory of the European Union and established under the "Habitat" 92/43/EEC and "Birds" 2009/147/EC Directives, with the aim of ensuring the long-term maintenance of natural habitats and endangered or rare species of flora and fauna at Community level.
Once fully operational, the Natura 2000 network will consist of Special Areas of Conservation (SAC) established by Member States, which will include both the SCIs under the "Habitats" Directive and the Special Protection Areas (SPA) under the "Birds" Directive.
The areas that make up the Natura 2000 network are not rigidly protected reserves where human activities are prohibited: the Habitats Directive aims to guarantee the protection of nature while also taking into account economic, social and cultural requirements, as well as regional and local characteristics.
In fact, the "Habitats" Directive recognizes the value of all those areas in which the age-old presence of mankind and man's traditional activities has enabled a balance to be maintained between human activities and nature. For example, many of the now rare and endangered animal and plant species are linked to rural areas and the survival of these species requires the continuation and enhancement of traditional activities like grazing or non-intensive agriculture. The title of the Directive itself specifies the goal of conserving not only natural, but also semi-natural habitats (such as areas of traditional agricultural, woods, pastures, etc.).
Another innovative part of the European conservation policy is recognition of the importance of several landscape elements that play a connecting role between wild flora and fauna. Member States are invited to maintain or, where necessary, develop these elements in order to improve the ecological coherence of the Natura 2000 network. In Italy, the SCIs and SPAs currently in the stages of final approval cover a total of 20% of the national territory.

The LIFE Programme is a financial instrument set up by the European Union to support environmental and nature conservation projects throughout the Union, as well as in some third countries, which are neighbouring and/or candidates for entry into the European Union. Since 1992, LIFE has co-financed more than 3,000 projects, allocating over 2 billion euro to environmental protection.
The main objective of the "Nature and Biodiversity" LIFE programme is to contribute to the conservation of species or habitats of Community interest within the territory of the European Union.

For further information on Natura 2000 sites in Italy, please refer to the specific web pages on the Ministry of the Environment and Protection of Land and Sea website (http://www.minambiente.it), while for the Natura 2000 network in other European Member States, please refer to the European Commission website on nature and biodiversity (http://ec.europa.eu/environment/nature/index_en.htm).

Italy has adopted the "Birds" Directive through Law n. 157, dated 11 February 1992 "Norms for the protection of warm-blooded fauna and for the regulation of hunting" and the "Habitats" Directive through Presidential Decree n. 357, dated 8 September 1997 and the subsequent Presidential Decree n.120, dated 12 March 2003, which delegates implementation to the various institutional authorities (State, Regions and Independent Provinces). More recently (2010), its own **National Strategy for Biodiversity** was approved, thus acquiring an important tool for ensuring effective integration between the development objectives of the country and the protection of its priceless heritage of biodiversity. In fact, it should be noted that our country features a remarkable variety of environments and contains a wealth of species and habitats, among the most significant in Europe, both in terms of the total number and the high rate of endemic species (found exclusively in a given territory). This is both for

bio-geographic reasons (geological nature, extension in latitude, central position in the Mediterranean basin, peninsular formation, presence of islands and archipelagos and numerous Alpine and Apennine elevations) and historical and demographic reasons (the millenarian presence of man, high population density).
In terms of the total number of species present in Europe, Italy has over 30% of the animal species and nearly 50% of plant species, all over a surface of approximately 1/30 of that of the whole continent.
More in detail: the fauna is estimated to include over 58,000 species, of which approximately 55,000 are Invertebrates (95%), 1,812 are Protozoa (3%) and 1,265 are Vertebrates (2%), with an overall incidence of endemic species of approximately 30%.
The flora consists of more than 6,700 species of vascular plants (of which 15% are endemic), 851 species of Mosses and 279 Hepatics. With regard to species of Fungus, there are about 20,000 known species of *Macromycetes* and *Myxomycota* (fungus species visible to the naked eye)[8].

The National Strategy for Biodiversity focuses on the following strategic objectives:

1. By 2020, to guarantee the conservation of biodiversity, meaning the variety of living organisms, their genetic variability and the ecological complexes of which they are a part, and ensure the protection and the reinstatement of the ecosystem services, in order to ensure their key role for life on Earth and for human well-being;
2. By 2020, to substantially reduce the impact of climate change on biodiversity within the national territory, by defining the necessary measures of adaptation relative to the induced changes and the mitigation of their effects, as well as increasing the resilience of the natural and semi-natural ecosystems;
3. By 2020, to integrate the conservation of biodiversity into economic and sectorial policies, also including employment opportunities and social development, by reinforcing the understanding of the benefits of the ecosystem services resulting from it and the awareness of the costs of losing these.

With regard to the identification of the sites to be included in the Natura 2000 network, in a first phase, the Ministry of the Environment has promoted and coordinated the creation of a computerized census of sites of Community Interest and of National and Regional Interest, through a specific research programme called "Bioitaly", leaving the subsequent phase of designation and updating to the individual Regions and autonomous Provinces.

The Tuscan Regional Authority has approved the delimitation of boundaries of the sites identified in the Bioitaly Project, and with Regional Council Resolution n. 342, dated November 10, 1998 and Tuscan Regional Law n. 56/2000 (Regulations for the conservation and protection of natural and semi-natural habitats and of wild flora and fauna), it equipped itself with a regulatory tool for protecting biodiversity, acknowledging the strategic role of the sites of Community, National and Regional Importance. This law identifies certain types of habitats and species considered to be of regional interest but not included in the annexes of the EU directives. In this context, the different types of sites (pSCI, SPA, SRI, SNI) were collectively classified as Sites of Regional Importance (SRI). The term "Sites of Regional Importance" therefore indicates those classified as Sites of Community Importance (SCI), Special Protection Areas (SPA) and the other sites, classified exclusively as sri (sites of regional interest). Tuscan Regional Law n. 56/2000 extends the rules pursuant to Presidential Decree 357/97 and its subsequent amendments or revisions to all Sites of Regional Importance (SRI).
It should also be noted that, in accordance with EU and national measures, the Tuscan Regional Authority has explicitly outlined the main conservation measures to be adopted in the Sites of Regional Importance through the approval of Regional Decree n. 644/2004 and has identified a list of shared minimum criteria for drafting conservation measures relative to special areas of conservation (SAC) and special protection areas (SPA), through the approval of Regional Decree n. 454/2008.
In the last five years, the Tuscan Regional Authority has finally drafted a "Regional Biodiversity Conservation

Action Plan", in keeping with the provisions of the National Strategy for Biodiversity and in collaboration with the WWF Italy. The process of drafting the Action Plan, which was launched in 2008 and ended in December 2011, saw numerous participants involved, both institutional (Regional Departments, Provincial Authorities, Nature Reserve Managing bodies, Universities, Research Institutes, State Forestry Department) and non-institutional (Environmental Protection Associations, Companies and technicians of the Sector).
Based on the results of the work carried out, the Regional Authority has defined the **Regional Biodiversity Strategy** currently being approved as an integral part of the Regional Environmental and Energy Plan (REEP 2013-15)[9]. Some of the key goals of the plan include "B. Protecting and promoting Territorial Resources, Nature and Biodiversity", and the detailed goals "B. 1 Increasing usability and sustainable management of the protected areas and preserving terrestrial and marine biodiversity".
The knowledge base for the Regional Strategy consists of two Nature Directories: *ReNaTo* (Natural Repertoire of Tuscany), relating to the flora, fauna and vegetation in the territory of Tuscany, edited by the "La Specola" Natural History Museum at the University of Florence and *BioMarT*, on vulnerable biocoenosis and rare species found in the sea off Tuscany, edited by the ARPAT (Tuscany Regional Environmental Protection Agency) Leghorn sea section and by the "La Specola" Natural History Museum at the University of Florence.

The importance of the wetlands and minor hydrographic network

A precise and detailed definition of the term "wetlands" can be found in the Convention on Wetlands of International Importance[10], signed in Ramsar (Iran) on 2 February 1971 by a group of countries, scientific institutions and international organisations. Article 1 of the Convention states that: "Pursuant to this Convention, the term wetlands includes swamps and marshes, peats or basins, whether natural, artificial, permanent or temporary, with stagnant or flowing fresh, brackish or salt water, including areas of seawater with depths, at low tide, that do not exceed six metres".
These areas are therefore a transition zone between water and land, and are characterized by the following distinctive features: high productivity (in terms of biomass); extremely rapid biological cycles; high biodiversity.
As a consequence of these characteristics, the numerous ecological functions performed by wetlands range from storage of carbon dioxide, phyto-purification, microclimate stabilization and the reduction of flood risks, to mention just a few[11].
Over time, the surfaces occupied by the wetlands have been progressively reduced to make room for crop cultivation and urban expansion (industry, building, infrastructures). It is estimated that, in Roman times, the area occupied by wetlands in Italy amounted to 3,000,000 ha (corresponding to about 10% of the national territory), while the currently represent only 2% of the national territory (although, on the other hand, they host to 31% of the species belonging to Italian birdlife[12]).
This reduction can be attributed to the repeated attempts to reclaim land, running from the Roman period right up to the 1960s, in order to accommodate the expansion of agriculture and urbanisation, for industry and infrastructures in what are particularly attractive areas: wetlands are, by nature and origin, concentrated in flat, fertile lands, where the introduction of machinery has greatly facilitated these profound territorial changes. Alongside the land reclamation, attempts were also made to reduce the risk of flooding by regulating watercourses and sanitary rehabilitation; given that the vast marshlands were known for their unhealthy conditions (we only have to think back to the spread of malaria).
In this changed environment, even the small wetlands (IAP - Important Areas for Ponds) not included in the Ramsar Convention constitute an important resource for the conservation of biodiversity, hosting around 200 species protected by European, national and/or regional legislation, including approximately 80 species of aquatic birds,

60 species of aquatic plants, over 20 species of amphibians, more than 15 species of aquatic invertebrates, five species of reptiles, three species of mammals and one species of fish. These have a fundamental function of connectivity between freshwater habitats, since they can serve as "stepping stones" for many migratory species or dispersing species[13].

With regard to the minor hydrographical network (with explicit reference in this context to the Apennine waterways, which are mostly torrential), these undoubtedly play a role in the conservation of biodiversity.
The mountain streams involved in the project (as well as most of the hillside ones) in the Prato and Bologna territories are located in areas that are particularly suited to protecting the species that they host, since they are situated on steep slopes with very few roads and are sparsely inhabited. In fact, urbanisation and industrial development in the Province of Prato, both in the distant past and more recently (from the post-war period onwards), has been concentrated in the Bisenzio valley and particularly in the vast flat area where the Bisenzio, the Ombrone Pistoiese and Arno rivers merge together. Similarly, urbanization, industrial and artisan activities in the Bologna area developed mainly in the lower Reno valley.
Along with the gradual depopulation of mountain areas, this has made it possible to maintain species and habitats of conservation interest present in the Apennines in satisfactory ecological conditions and provide interesting opportunities for conservation measures to protect them.

THE "WATER SCIS" LIFE PROJECT - ORIGIN, AIM, PLANNED ACTIONS AND EXPECTED RESULTS

A combination of factors led to the idea of participating in the LIFE 2007 public tender with the "Water SCIs" project, including:

- The availability of reliable, in-depth and up-to-date knowledge on the conservation status of species and habitats present in the respective territories of jurisdiction, gathered by the Provincial Authority of Prato and by the Lakes Suviana and Brasimone Natural Park (Park of Lakes) during their official activities (for example, the collection entitled "Biodiversity in the Province of Prato" and the ARCA project: http://mapserver.provincia.prato.it/prv_po/arca/index.php);
- The presence, both in the Province and in the Lakes Suviana and Brasimone Natural Park, of a working group consisting of competent and motivated people, open to cross-institutional collaboration with the local administration of the neighbouring territories;
- The need, expressed by the European Commission and by the Tuscan Regional Authority (Communication Protocol n. 38338/2004), to protect species of fish that are insufficiently represented in the Natura 2000 Network sites attributed to the Mediterranean Bio-geographical Region, including: *Lampetra planeri, Leuciscus lucumonis, Rutilus rubilio, Barbus plebejus, Padogobius nigricans,* ***Cottus gobio*** and the consequent invitation, addressed to the Provincial Administrations concerned, to *express their opinion regarding the designation and consequent inclusion of the SRI (sites of regional importance) and protected areas listed above* (including the "**Alto Carigiola**" Protected Area and the **Acquerino-Cantagallo Nature Reserve** – Ed.) *in the European Ecological Network Natura 2000;*
- The need to place the wetlands in the Prato plains area under greater protection, as sites of strategic importance for bird fauna, particularly during migration phases, due to their geographical location (close to the Apennine mountain range);
- The willingness of the local administrations involved to accept the challenge of participating in a selective and competitive procedure at European level in the nature conservation field, and to take charge of the

subsequent operational and administrative management of the project, if we won the tender;
- The previous experience gained by the staff of the Provincial Authority of Prato in managing a community project, in the role of beneficiary coordinator (LIFE project NAT/IT/007170 "Habio").

This was the inspiration for the project, based on a proposal made to the Tuscan Regional Authority to designate two new sites as part of the Natura 2000 network. The aim was to provide a greater guarantee of protection to several precious Apennine areas (and the waterways and species/habitats present within them) and to a few minor wetlands remaining in the plain, in this second case, by expanding the already existing "Ponds of the Florentine Plain" SCI/SPA/SRI and making it the current "Ponds of the Florentine and Prato plain" SCI/SPA/SRI. Within these newly established sites, we proposed implementing a series of environmental improvement measures to make them more suitable for bird populations to stopover and nest and for the survival of amphibian species, the local populations of which showed a marked decline trend.

In fact, the project's ultimate goal is "to improve the conservation status of the Species of Community Interest that are not adequately represented or are exposed to risks in the upper Apennine area and in the plain surrounding Prato, namely:
- Various species of birds protected by the EU "Birds" Directive (79/409/EEC, later replaced by 2009/147/EEC) and the amphibian *Triturus carnifex*, included in Annexe II of the EU "Habitats" Directive (92/43/EEC), present in the wetlands of the Prato plain between the Arno and Ombrone Pistoiese rivers;
- The White-clawed crayfish *Austropotamobius pallipes**), species with a high conservation priority, and the fish species, *Cottus gobio*, both included in Annexe II of the EU "Habitats" Directive (92/43/EEC) and present in the streams of the Tuscan-Emilian Apennines.

To achieve this goal, we planned the following project activities:
a) Conservation measures for the target bird and amphibian species:
- Expansion of the surface area of the current wetlands in order to create suitable habitats for feeding, nesting and wintering of the target species of birds;
- Recovery of lake shores and creation of buffer zones around the wetlands to mitigate the negative impact of various human activities and infrastructures;
- Improving the management of wetlands in the Plain, their water levels and lake shores;
- Expansion of the "Ponds of the Florentine plain" Special Protection Area (code IT5140011) to include the main wetlands present in the territory of the Province of Prato, useful for the conservation of species of birds and amphibians protected by the EC Directives;
- Approval of a Natura 2000 Management Plan for the new SPA, in order to ensure a long-term commitment towards the conservation objectives.

b) Actions related to the target species of fish and astacidae:
- Restoring water continuity in several Apennine streams by creating fish ladders to encourage the spread of *C. gobio*;
- Facilitating the growth of fish and invertebrate populations by creating sites that are suitable for natural reproduction (*in-situ*) and hatchery reproduction (*ex-situ*);
- Expanding the "Lakes Suviana and Brasimone" pSCI (code IT4050020) to include important areas for the survival of the *A. pallipes** and *C. gobio* populations of the Tuscan-Emilian Apennines, as well as other important habitats and species of Community interest.

c) Actions to raise public awareness and promote the conservation of the target species:
- Environmental education initiatives aimed at schools and citizens in general, designed to provide knowledge of the target species and habitats and their ecology, as well as the actions put into place thanks to the "Water SCIs" LIFE project to improve their conservation status;
- Printing and distributing publications about the project (brochure, the fisherman's handbook, final publication);
- Installing Notice Boards and setting up a website to raise public awareness of the importance of protecting the wetlands in the Plain and the upper Apennine waterways, for the conservation of Species of Community Interest.

These actions aimed to achieve the following results:
a) With reference to the conservation of target species of birds and amphibians:
- Stabilizing the presence of bird species of Community interest in the Prato plain, to be monitored through the number of wintering species, recurrent migratory species, nesting species, and the number of specimens for species sampled before, during and at the end of the project;
- Redeveloping 10 ha of wetlands, in order to allow for the conservation of target species of birds and amphibians;
- Redeveloping 6.5 ha of lake shores, for nature conservation purposes;
- Enlarging (by at least 300 ha) the "Ponds of the Florentine plain" SPA (Nat2000 code: IT5140011);
- Obtaining approval for the Natura 2000 Management Plan for the enlarged SPA.

b) With reference to the conservation of fish and astacidae target species:
- Creating fish ladders to facilitate the spread of *C. gobio*;
- Increasing the numbers and reproductive ability of the *A. pallipes* and C. gobio* populations in the Tuscan-Emilian Apennines;
- Constructing a fish hatchery for the reproduction of target fish and astacidae species;
- Obtaining approval for conservation Action Plans for the target species;
- Enlarging (by at least 2,500 ha) the "Lakes Suviana and Brasimone" SCI (Nat2000 code: IT4050020).

c) With reference to the "public awareness" actions:
- Raising awareness about the key role played by residual wetlands in the plain around Prato and the watercourses in the upper Apennines, aimed at the conservation of Species of Community Interest.

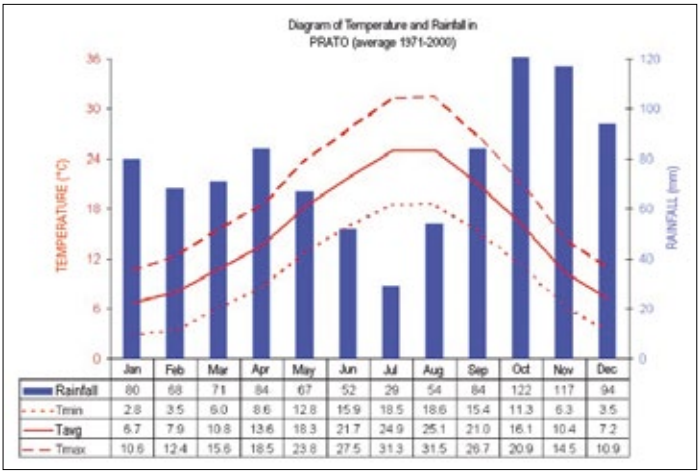
CLIMATE OUTLINE

General information

The territory involved in the project is characterized by a rather complex topography and orography, which play an important role in defining the climate around the whole basin.
The orientation of the Apennine mountain range on the one hand ensures protection from the cold and normally dry currents coming from the north-east and, on the other hand, a particular response to the damp currents from the south-west. Tuscany's climate varies, ranging from typically Mediterranean to moderately hot and cold, mainly following the gradients related to the altitude, latitude and distance from the sea. The seasonal breakdown of average rainfall identifies the territory in question as a sub-coastal system, with the maximum levels in autumn and spring and the minimum in summer.
In addition to the presence of the Apennines, the climate in the Province of Prato is influenced by the north-south orientation of the Bisenzio river, which flows in the valley of the same name, enclosed between the hills of Calvana (on the left bank) and those of Monteferrato (on the right bank) channelling the winds of the northern quadrants over the plain. The flat area, where the development of settlements and industry were focused, is part of the broader floodplain (called the Florence-Prato-Pistoia plain) and was probably created about 50,000 years ago in the Middle Pleistocene period where the Bisenzio and Ombrone Pistoiese rivers join the Arno. At the time, the plain must have been a waterlogged basin punctuated by stretches of shallow water, alternating with patches of vegetation, swamps and streams, meandering in places and in other places fragmented into dozens of little still minor watercourses[14].

Temperature and precipitation

For the characterization of temperature and rainfall in the Prato plain, reference was made to the Centre "Florence-Peretola" Observatory. Average annual monthly temperature trends are shown in the charts below.



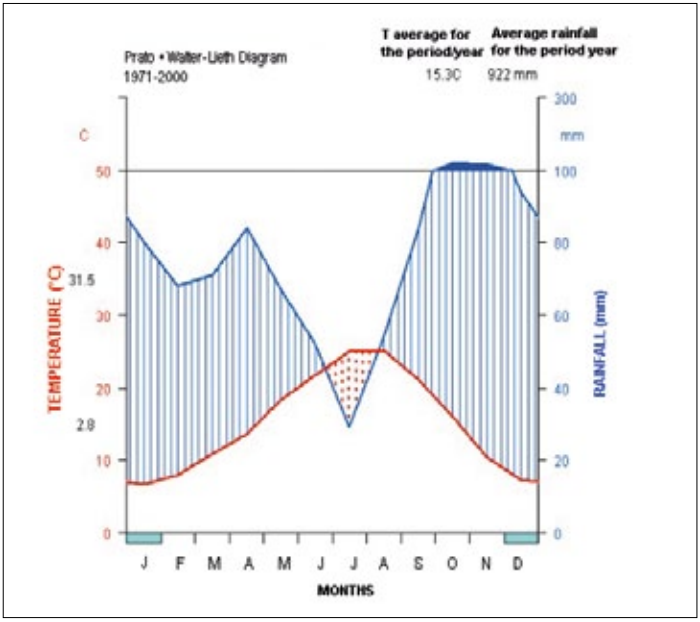
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Rainfall	80	68	71	84	67	52	29	54	84	122	117	94
Tmin	2.8	3.5	6.0	8.6	12.8	15.9	18.5	18.6	15.4	11.3	6.3	3.5
Tavg	6.7	7.9	10.8	13.6	18.3	21.7	24.9	25.1	21.0	16.1	10.4	7.2
Tmax	10.6	12.4	15.6	18.5	23.8	27.5	31.3	31.5	26.7	20.9	14.5	10.9

Tab. 1 – Diagram of temperature and rainfall in Prato (data from the regional hydrological service).

The trend, with a rather irregular pattern, shows the highest maximum monthly temperatures of just over 31° C in the months of July and August and minimum temperatures in the month of January (Min. temp. = 2.8°C). These values indicate pronounced seasonal temperature variation with cold winters and hot summers, identifying this lowland area with a temperate sub-continental climate type (annual temperature variation of over 19°C).

From the average monthly rainfall trend you can see that the autumn months have the highest rainfall, with a seasonal average of around 100 mm of rain.

Relative humidity was quite high both in summer and in winter, with an average value of 70.25%.
The Walter-Lieth climogram represents the trend of monthly rainfall and average monthly temperatures in a single graph, making it easy to see the main features of a climate system, especially relative to any dry periods. When building the climogram in ordered sequence, the following are represented: I) to the right is monthly rainfall in millimetres and II) to the left are the monthly average temperatures in degrees Celsius; III) the x-axis indicates the months of the year, from January to December.
The diagram shows a dry period in the graph area bordered by the intersections between the rainfall and temperature curves; the resulting water deficit is proportional to the duration and intensity of the dry period. From a bioclimatic point of view, it is important to know when this period occurs during the year. At the mid latitudes, where plant species have their dormant period in winter, a dry period during the winter months has no effect; conversely, a dry period in summer (as in the regions with a Mediterranean climate, where the minimum rainfall coincides with the hottest season) has considerable effects on vegetative growth and leads to the selection of species with suitable anatomical and physiological adaptations.



Tab. 2 – Walter-Lieth climograph diagram. The area delimited by the intersections of the two curves highlights the dry period.

For the territory of Prato, the diagram represented in Tab. 2 shows a regular dry period in the month of July; in fact, we can see how in this period the minimum rainfall coincides with the maximum temperature.

The Apennine area had more rainfall than the plains (with figures of over 1,000 mm per year everywhere), despite their similar trend: rainfall is concentrated in autumn (October-December) and spring, while minimum rainfall occurs during the summer. There is a typically mountain climate in the Apennines, where there is often snow in the winter. In summer, the area is often affected by moderate thunderstorm activity, of a thermal-convective type, which sometimes also deviated into the lowland area.

Geological overview

Geology

For geologists, the northern Apennines represent an orogenetic mountain range of recent formation (in geological terms): its most important stages in its structure and elevation took place over the last 35 million years. The geological edifice of the northern Apennines, along with the Alps, is part of that important and complex orogenetic belt known as alpine, which extends from Spain/North Africa (Gibraltar) through the Alps and the Himalayas, as far as Indonesia.

According to theories deriving from lithospheric plate tectonics, this orogenetic belt in the Mediterranean area is the result of the convergence (Late Cretaceous – Mid Eocene) and subsequent collision (Late Eocene - Oligocene) of the Paleo-African continent (or the Adria microplate) with the European continent, which, due to the subduction of the oceanic crust below the European margin, caused the disappearance of the Jurassic Ocean, namely the Tethys Ocean, that separated them.

Today's forms of elevation in this region are the result of a complex series of geological events that occurred over tens of millions of years (=My). There were two particularly important stages in the geological history of the northern Apennines:

1) 27-10 My: Formation of the Apennine orogenic range as a result of compression phenomena that led to the overlapping of rocky masses (structural units or strata) from the Mesozoic-tertiary period pertaining to different areas of sedimentation (paleo-geographic domains), with distances between each other of 150 miles or more from the place in which we find them today (non-native units).

2) 10 My – to date: Elevation and relaxation of the orogenic edifice with the formation of valleys (graben) and mountain ridges (horst), mostly oriented in parallel with the development of the range (NW-SE), and delimited by systems of fractures (faults).

The geological formations present are predominantly impervious, made up of clay, marl, shale clay, marly limestone and compact sandstone. The distinctly permeable part of the basin does not exceed 5% of the entire surface. Overall, the rocks making up the Arno basin are easily eroded. The colouring of the flowing waters, which is usually yellowish, is itself an indication of a strong suspended load stream capacity. This results in a strong tendency towards denudation of the basin, despite the fact that Tuscany, as a whole, is one of the regions richest in wooded areas with respect to the overall agricultural and forestry surface area.

The complex tectonic history of Tuscany is also reflected in a wide variety of geological formations that emerge in the basin of the River Arno. They are mostly sedimentary, as they are linked to the widespread deposition phenomena relative to an extensional dynamic, and are also subject to magmatic and metamorphic phenomena, which can be attributed mainly to the units linked to the compressive phases. Due to the convergence between the African and European plates, large masses of oceanic crust and its relative sedimentary cover (accumulated over millions of years) were deformed and pushed over portions of continental crust, also covered by thousands

of meters of marine sediments deposited over millions of years. Once these two rock masses had overlapped, when the horizontal forces due to the convergence of the plates were exhausted, the rock masses were impacted by the development of faults and fractures that led to the formation of a basin, which, when filled with geologically recent river and lake sediments, gave rise to the Prato plain[14].

Tectonic Units

Studies conducted in the territory of the Province of Prato made it possible to establish the presence of rocks that emerged from at least five different overlapping tectonic units, to form the orogenic edifice, characteristic of this part of the northern Apennines. Hence, the rocks observed come from sequences, originally deposited in contiguity, that were deformed and dislocated from their original position by the horizontal tectonic thrusts, thereby forming tectonic units (or "scaglie"- meaning thin layers like scales or flakes) stacked upon each other. The tectonic units recognizable in the Province of Prato are, from bottom to top: the Castiglion dei Pepoli unit, the Cervarola-Falterona unit, the Tuscan Nappe unit, the Monte Morello unit and the Sestola-Vidiciatico unit. Above these units are the sedimentary deposits that accumulated during or after the last phases of deformation: river-lake deposits in the Prato plain, terraced deposits, etc.[14].

Hydrographic overview

The areas involved in the project are partly in the catchment sub-basin of Valdarno Medio, a part of the national hydrographic basin of the River Arno, and partly in the catchment basin of the River Reno. The Valdarno Medio sub-basin, which includes a large part of the territory of the Province of Prato, originates downstream of Pontassieve, including the sub-basins of the River Bisenzio, the right bank of the River Ombrone and the left banks of the River Greve and the River Ema (Fig. 1). The confluence of the Arno and Ombrone rivers determines the closure of the sub-basin. The basin of the River Reno, on the other hand, affects the high part of the Apennines in the Province of Prato (the Limentra torrent in the Municipality of Cantagallo and the Setta stream in the Municipality of Vernio) and the territory of the Province of Bologna (Fig. 2).

Fig. 1 – Catchment basin of the River Arno.

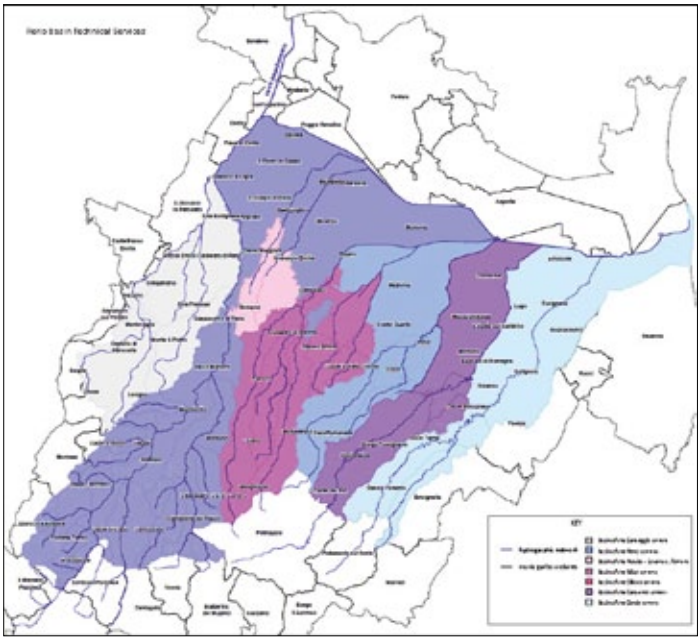


Fig. 2 – Catchment basin of the River Reno.

THE NATURA 2000 SITES ESTABLISHED BY THE "Water SCIs" LIFE PROJECT

Among the expected results, the Community project included the enlargement of the "Ponds of the Florentine plain" SPA (Nat2000 code: IT5140011) and the "Suviana and Brasimone Lakes" SCI (Nat2000 code: IT4050020) to encompass the areas affected by environmental improvement interventions. Right from the initial phases of the project, based on the detailed naturalistic studies carried out between 2000 and 2008, the Provincial Authority of Prato and the Tuscan Regional Authority were able to define the boundaries of the new Nature 2000 areas.

The SCI/SPA/SRI "Ponds of the Florentine and Prato plain" (Natura 2000 code: IT5140011), in the territory of the Province of Prato, consists of three separate core areas (A, B, C in the figure below) located near the course of the River Ombrone, on the left bank, to the south west and to the west of the town of Prato, on land situated primarily in the administrative territory of the Municipality of Prato and are also subject to the Municipality of Poggio a Caiano. The current surface area of the site is the result of the enlargement, by about 557 ha, of the previous "Ponds of the Florentine plain" SCI/SPA/SRI (established in 1998 by Regional Council Resolution n. 342), pursuant to Regional Council Resolution n. 80/2009, based on a proposal put forward by the Provincial Authority of Prato.

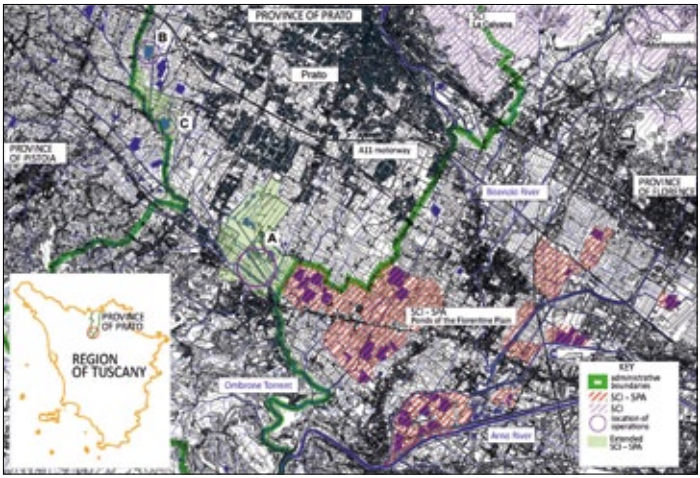


Fig. 3 – Location of the operations included and the proposed boundaries for the enlargement of the "Ponds of the Florentine plain" SPA (Natura 2000 code: IT5140011).

The site currently covers a total of about 1,902 hectares, located at an altitude of between 32 and 91 m above sea level. The most important infrastructure is the A11 Motorway (Firenze-Mare), located in the north-eastern area of the site. The "Cascine di Tavola" Nature Reserve of Local Interest is entirely included within the Prato part of the SCI/SPA/SRI. The SCI/SPA/SRI IT5140011 is included in the Mediterranean Bio-geographical Region.

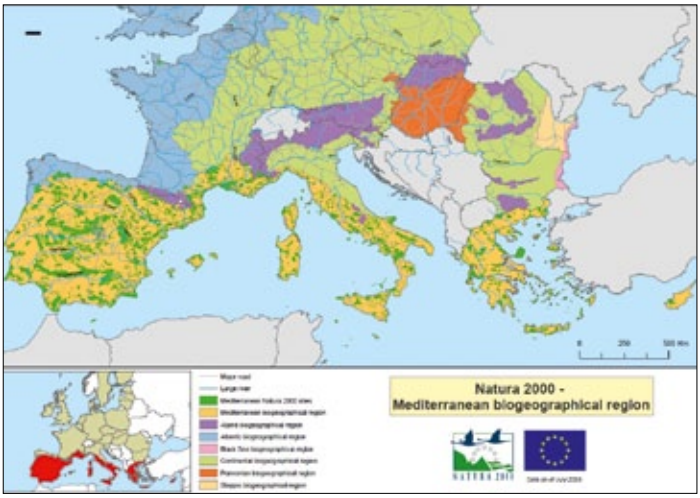


Fig. 4 – Map of the Mediterranean Bio-geographical Region

The "Prato Apennine" pSCI (Natura 2000 code: IT5150003) is entirely included within the territory of the province of Prato, occupying a total surface area of 4,191 hectares of mountainous territory, and is characterized by well-maintained forest areas.

The ecosystems of the watercourses, particularly of the Limentra di Treppio, Carigiola, Trogola and Canvella torrents and the upper course of the Setta and the Bisenzio rivers, have an excellent conservation status in terms of water quality and the presence of shrub and tree riparian vegetation, as well as the herpetofauna, astacofauna and ichthyofauna that it hosts. The site was designated by Regional Council Resolution n. 80/2009 based on a proposal put forward by the Provincial Authority of Prato.

The site includes the "Alto Carigiola e Monte delle Scalette" Nature Reserve of Local Interest, including the territory of the Municipalities of Vernio and Cantagallo and the "Acquerino-Cantagallo" Provincial Nature Reserve, which is entirely inside the territory of the Municipality of Cantagallo. The western and northern boundaries of pSCI IT5150003 coincide respectively with those of the "Tre Limentre-Reno" pSCI (Natura 2000 code: IT5130009), in the Province of Pistoia and those of the "Suviana and Brasimone Lakes" pSCI (Natura 2000 code: IT4050020), in the Province of Bologna, forming a vast portion (approximately 15,453 hectares) of Apennine territory entirely protected by the "Habitats" Directive.

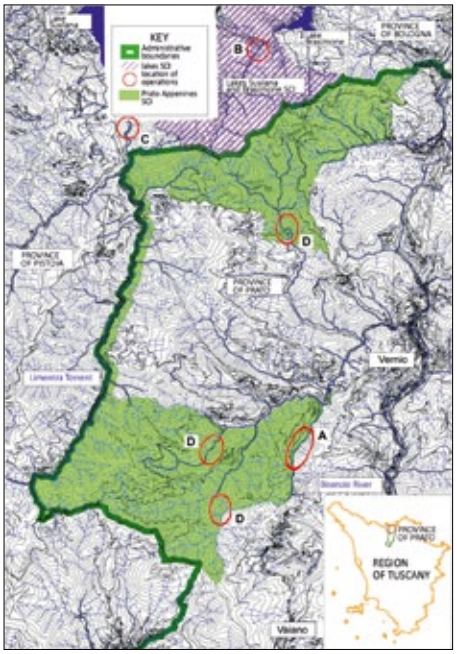


Fig. 5 – Location of the operations included and proposed boundaries for the designation of the "Prato Apennine" SCI (Natura 2000 code: IT5150003).

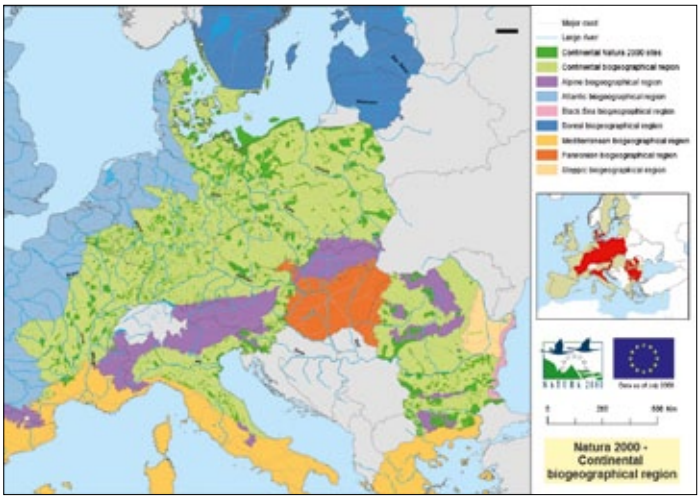


Fig. 6 – Map of the Mediterranean Bio-geographical Region.

Preliminary analysis of the target species

Analysis of the presence, numbers and distribution relative to the target species in areas involved in the project (as well as their state of conservation and the possible presence of native and alien competitors) constitutes an essential prerequisite for the correct planning of any concrete conservation action. In fact, a sufficiently extensive knowledge base on the ecological conditions of the species and habitats present and the dynamics involved, including any threats or pressure factors, is essential for any intervention in the territory.

The territory included in the project, in the case of the Prato plain, consists of an area which has been strongly affected by human activity and which runs into the Florentine and Pistoia plains. It is characterized by the presence of highly fragmented semi-natural areas (residual agricultural areas and artificial wetlands, some of which are used for hunting purposes) in the context of a highly urbanised area. Despite the limited naturalistic value of these elements when taken individually, the ecological importance of the area should be seen in the context of an ecological network, the primary, secondary and potential nodes of which combine together to form a single functional unit. This area is, in fact, very important for the reproduction and migration of birds. It has been included in the European network of I.B.A (Important Bird Areas) and its role is one of national importance for the wintering of the Little Grebe (*Tachybaptus ruficollis*), the Cattle egret (*Bubulcus ibis*), the Squacco heron (*Ardeola ralloides*), the Common Moorhen (*Gallinula chloropus*) and it is of regional importance for the White heron (*Ardea cinerea*)[15].

The Prato plain wetlands are, therefore, home to numerous species, which also vary considerably in number and abundance from year to year, depending on the size of the migratory bird populations passing through. Thus, from the start-up phase of the project and based on emerging evidence during preliminary studies, we decided, by implementing the environmental improvement actions anticipated, to focus mainly on species included in Annex I of the "Birds" Directive (2009/147/EC) observed (sometimes sporadically) in the territory, with particular regard to the following: the Ferruginous Duck (*Aythya nyroca*), the Night heron (*Nycticorax nictycorax*), the Little egret (*Egretta garzetta*), the Black-winged Stilt (*Himantopus himantopus*), the Kingfisher (*Alcedo atthis*) and the Red-backed Shrike (*Lanius collurio*).

The project also aims to improve the conservation status of Species of Community Interest which are not present

in sufficient numbers or are exposed to risks in the upper Apennine area and on the plain surrounding Prato, with specific reference to the following target animal species: the Italian crested Newt (*Triturus carnifex*), the White-clawed crayfish (*Austropotamobius pallipes*) and the European bullhead (*Cottus gobio*), protected by the "Habitats" Directive (92/43/EC).
The implementation of preliminary analysis was entrusted to a group of companies including StudioSilva in Bologna, Bioprogramm in Padua and Comunità Ambiente in Rome, following a public tender. As part of these studies, a standard protocol was developed for the technical and scientific monitoring of the target species and was used to assess the conservation status of the populations of these species throughout the project cycle and the effects of the active conservation measures implemented.
Similarly, on the Bologna side of the Apennines, preliminary investigations of *C. gobio* and *A. pallipes* were conducted by Dr. Giuliano Gandolfi, an ichthyologist commissioned by the Parco Regionale dei Laghi Suviana e Brasimone (Lakes Suviana and Brasimone Natural Park), and by a team of experts from the Department of Biological, Geological and Environmental Sciences (BiGeE) of the University of Bologna.
The bibliographical studies and preliminary campaign surveys were carried out in the period from November 2009 to June 2010. The results, recorded in the summary that follows, were included in a special report, as well as in paragraph 2.1.3 of the Action Plan for the conservation of *A. pallipes* and *C. Gobio* approved by the Lakes Suviana and Brasimone Natural Park. Both documents can be downloaded from the project Internet website: http://life.provincia.prato.it/.

Ichthyofauna

EUROPEAN BULLHEAD

Fig. 7 – *Cottus gobio* (Linnaeus, 1758)
Order: SCORPENIFORMES Family: COTTIDAE

Systematics and identification

Cottids, which are only found in the northern hemisphere and have a circumpolar distribution area, are usually benthic animals with a fusiform body and a big head, often compressed in the dorso-ventral direction. The eyes are positioned at the top of the head and are close to each other, and the mouth is wide. The skin is almost completely devoid of scales, but there are barbs or tubercles on the body, concentrated particularly on the head. They have two dorsal fins and the pectoral fins are usually large and fan-shaped. A lateral line along the sides is the typical fish sense organ, formed by a canal that runs along the side of the animal. In this family it is sometimes incomplete or interrupted in several points. The swim bladder is absent[16]. *C. gobio* is a very ancient member of European fish fauna and can be traced as far back as before the Pleistocene era[17]. Of the over 1,200 species of Scorpaeniformes, only just over 50 live in fresh water and the vast majority of these are Cottids (although this family does include a large number of marine species[18]). The European bullhead is a species that is small size, no more than 15 cm in length[19]. It has a rather wide mouth and prominent cheeks. Its colour varies from brown to greenish with darker marks spread over the entire surface of the body. This species only has a small number of barbs near the pectoral fins. If carrying out a superficial examination, the European bullhead may be confused with the Goby, given the general similarity between the two species, but the different shape of the ventral fins (which are divided in the European bullhead) and the total lack of scales, in addition to its slightly larger size, make this species unmistakable on closer examination.

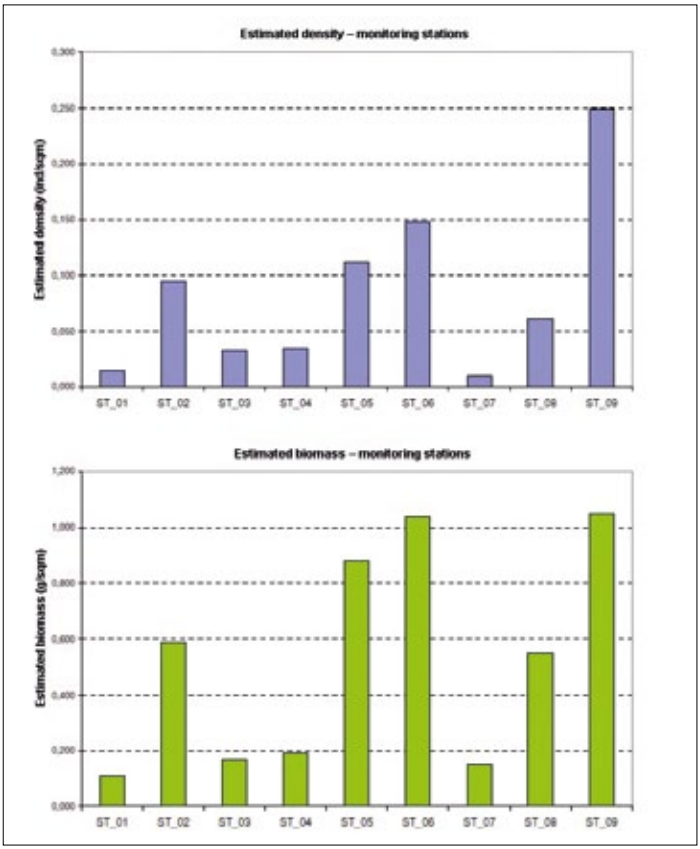
Distribution and presence in the study area

The European bullhead is a widely distributed species in Europe and is commonly found from the Pyrenees to the Urals and from Scandinavia to Northern and Central Italy[20]. As far as Italy is concerned, the European bullhead, despite its patchy distribution, is present throughout the Alps and also in the Apennine watercourses, on both the Tyrrhenian and the Adriatic sides, speaking of which it is useful to remember that this species is considered native to both of the two most important Italian ichthyofaunistic areas. This is actually an approximation because there is currently insufficient data with which to attribute the certain autochthony of the species to one of the two areas. Scientific research based on molecular analysis is currently under way with the aim of clarifying the geographic origin of the species and its distribution.
In the territory of the Province of Prato, the European Bullhead has been observed both in the sub-basin of the Limentra and Setta torrents, which are both part of the Reno River basin (Adriatic side), as well as in the sub-basin of the Bisenzio river, belonging to the basin of the Arno river (Tyrrhenian side). This information has been integrated from censuses conducted in the field, carried out at nine stations placed on the main bodies of water present in the study area, by means of the "electrofishing" technique, using a fixed electrical stunner, powered by pulsed direct current and modulated voltage (0.3-3 Amp, 150-600 Volt, 2,500 W) and a shoulder-carried electrical stunner, powered by pulsed direct current and modulated voltage (3.8-7 Amp, 300 - 500 Volt, 1,300 W).
The species was found in all the stations investigated, with biomass and density estimated as variable. The tables below show the values detected at the nine survey stations.
The populations studied in the watercourses of the Bologna side (conducted by monitoring 10 sampling points), turned out to be very articulate and abundant, with the exception of the station along the Brasimone river in the locality of Lavaccioni di Sotto, where there was evidence of a population that was unstructured and numerically small. A single specimen of European bullhead was discovered along the stream of Bago. It was unclear whether or not this was a one-time happening, due to the activity of reshuffling of the fish fauna, or if a stable, structured population existed along the watercourse being investigated.

Presence of possible native or alien competitors

The competitors of the C. gobio are effectively all predators, specifically Ichthyophagi like the salmonid fauna that generally populate the same distribution areas. The information on the presence and number of competitors was extrapolated from the direct surveys performed in the field with the aim of monitoring and thus improving the target species' ability to spread, for *in-situ* and *ex-situ* reproduction. In the geographic area studied, the only salmonid present was the Sea trout, which, from a feeding point of view, is an opportunist. In fact, it eats whatever nature offers in the greatest amounts: mainly macro-invertebrates, but also small fish like the European bullhead. The literature makes it clear that the fish preyed upon account for only a small percentage of the food ingested by the trout[21,22], or even nothing at all[23]. Based on studies conducted in the Veneto region, specifically in the Piave river and its major tributaries in the province of Belluno, where the European bullhead is very well-distributed and abundant, it was noticed that the Marble trout greatly prefers ichthyophagi, compared to the Sea trout and to the specimens that are hybrids of the two species[22], while from studies carried out in province of Treviso, it appears that the sea trout and the rainbow trout have similar stomach contents[21].
As well as the predation factor, the European bullhead presumably also establishes a sort of feeding competition with the salmonids. In fact, the European bullhead feeds mainly on benthic invertebrates, small fish and fry, as well as the eggs of other species. For the proper protection and management of the European bullhead, special attention should therefore be paid to the repopulation of salmonids carried out for fishing purposes.
Confirming this, research carried out in the watercourses on the Bologna side led to the capture, in various circumstances, of non-native salmonids along the Brasimone river in the locality of Mangiamele and, in particular, of some mixed phenotypes, probably attributable to hybrids forms of Brook trout (*Salvelinus fontinalis*) mixed with Sea trout [*Salmo (trutta) trutta*] in varying degrees. These discoveries were interpreted as a clear sign of inadequate management of the repopulation carried out in the water course, probably due to propagation ma-

Tab. 3 – Estimated density and Biomass, 2010.



terials of questionable origin and poor quality.

To this end, we requested the Provincial Authority of Bologna to immediately suspend any fish fauna propagation not specifically authorized and not oriented towards the goals of the LIFE project, for the duration of the Project itself. Moreover, the Fish Commission of the Basin should be informed and the measures adopted should be inserted into the provisions put into effect by the Provincial Fish Plan. The suspension requested involved the Limentra di Treppio torrent and its tributaries, from the closing embankment of the river basin of Suviana to the regional border, and the stretch of the Brasimone torrent inside the perimeter of the Lakes Suviana and Brasimone Natural Park and its tributaries.

Conservation status in the study area

The conservation status of this species was estimated by taking into account the size of the populations, deduced both from the historical reconstruction of their presence and from direct censuses conducted as part of the "Water SCIs" LIFE project".

From the data collected at the various research stations on the Prato side, the species proved to have a good conservation in some stretches of the Apennine water courses.

As far as the Bologna side was concerned, on the whole, the populations of *C. gobio* found proved to be very

well-structured and abundant, highlighting what is considered to be a green conservation status, apart from some exceptions related to the stations at Lavaccioni di sotto, along the Brasimone torrent (amber conservation status), and at the Bago brook (red conservation status).

Astacofauna

WHITE-CLAWED CRAYFISH

Fig. 8 – *Austropotamobius pallipes* (Lereboullet, 1858)
Ordine: DECAPODA Famiglia: ASTACIDAE

Systematics and identification

A. pallipes poses considerable difficulties from a systematics point of view and has been the object of numerous taxonomic reviews, conducted mainly on the alpine populations[24,25,26]. Through the analysis of morphological and meristic features, the molecular analysis through alloenzymes and the evaluation of genetic variability, numerous different species and subspecies were defined in each specific case. From a general point of view, reference is currently made to an *A. pallipes species complex* characterized by various mitochondrial lines in different Italian bio-geographical areas[25]. The importance of the molecular typing of the various populations is particularly justified by the fact that, today, Italy is considered a "hotspot" of biodiversity for this taxon, at European level. The existence of four distinct haplotypes of A. pallipes in Italy, which are not supported by differentiation in terms of a nuclear genome nor, above all, are they informative with regard to a precise phylo-geographic distribution, has led to a systematic review of the taxon and the proposed existence of the single species of *A. pallipes*[27]. Here, in this volume, we will be using the traditional nomenclature[28] (Souty-Grosset et al., 2006).

A. pallipes is a medium-large sized species (maximum size of approximately 12 cm total length) and it varies in colour from to light to dark brown or olive green. The largest of its appendages are the pincers. These have multiple functions: capturing and manipulating prey, defence against predators, reproductive activities (mating and competition with other males), as well as intra and interspecies combat. This species must not be confused with the invasive species, *Procambarus clarkii*, present in numerous areas of Tuscany and characterized by its large size, lateral red-violet colour and almost black dorsal colour.

The White-clawed crayfish lives in mountain watercourses (up to a height of 1,260 m in southern Switzerland), thanks to its ability to resist at low temperatures. However, the temperature of the water must not go below 10°C, in order to allow its young to develop normally. The temperature also has a strong impact of the species' activities, which fall to almost zero during the winter. This species is very sensitive to chemical pollution and is, therefore, considered a good indicator of healthy water conditions[29]. The most important requirement of this species, from a chemical point of view, is that there be a minimum oxygen concentration of at least 6 ppm (a saturation value of 60% is optimal) and a quantity of calcium not inferior to 2.8 ppm. In fact, calcium is a decidedly limiting factor for the Crayfish, as it is necessary for hardening the exoskeleton after shedding. The activity of this species is predominantly nocturnal[30]. As for other freshwater crustaceans, nocturnal habits are considered an adaptation that allows them escape from predators whose hunting is based on sight, including several species of fish, birds and mammals. The natural shelters in which these macro-invertebrates take refuge, also from their conspecifics, are the roots of trees near the river banks, vegetal detritus and large pebbles. Mating

take place in autumn and it is possible to find egg-laying females from December to June. Every female retains the fertilized eggs (approx. 40-150) below her abdomen. Larval development is direct and the larvae remain stuck to the female until to the third stage, after which they leave and lead an independent life. Sexual maturity is reached after 2-3 years. The diet of this species, which plays a fundamental role in the food webs of the freshwater ecosystems[29], consists of animal matter, such as the larvae of insects, fish and other dead animals, as well as fresh vegetable matter, like moss, and debris.

Distribution and presence in the study area

The White-clawed crayfish is present throughout the Italian territory, with the exception of parts of Calabria and Puglia. The species is present in the northern part of the province of Prato, in the municipalities of Vernio, Cantagallo and Vaiano. The conservation status, estimated on the basis of both historical reconstruction of its presence and based on the direct censuses carried out as part of this project can be considered quite good, even though it currently has a limited distribution area.

Historical data available on the White-clawed crayfish was collected from the "Carta ittica (fish map) of the Province of Prato" updated as of 2008, from a technical report regarding the distribution of protected species (ex-Tuscan Regional Law n. 56, dated 6 April 2000) and by consulting local experts. An initial monitoring activity, performed using the technique of electrical fishing, gave poor results. This was followed by other monitoring activities, during the course of the project, carried out by expert staff through manual censuses.

In the territory of the Province of Bologna, the presence of the White-clawed crayfish proved to be limited to just a few stations, with highly fragmented distribution, in some cases of a puntiform nature, and numerically small. In particular, it must be noted that the species was not detected within the main branch of the Limentra di Treppio torrent, where the species had been common in the past[31]. This phenomenon, which is difficult to interpret without a solid foundation of data, can be generically traced back to the strong pressure of extraction to which the species was subjected in previous years, to changes in the hydrological regime of the watercourse with sudden large-scale flooding events or to the uncontrolled, over-densifying trout repopulation events, which probably led to considerable predation, especially of young specimens and adults in the shedding phase.

Lastly, the discovery of breeding grounds of *Aphanomyces astaci* or "crayfish plague", caused by the fungus *Aphanomyces astaci* (which came from North America with the import of the Signal crayfish *Pacifastacus leniusculus*) is of considerable importance. These breeding grounds were found along the brooks of Bago and Malsacco, minor tributaries of the Limentra di Treppio torrent, and were confirmed by the presence of several dead specimens resulting from this pathogen.

The presence of possible native or alien competitors

The competitors of *A. pallipes* are, as for the *C. gobio*, Ichthyophagi predators like salmonid fauna. The gathering of information relative to the presence and number of competitors was extrapolated from direct actions performed in the field, the purpose of which was monitoring, in order to improve the dispersion capabilities of the target species, for *in-situ* and *ex-situ* reproduction.

Young crayfish and shedding adults can be prey for Salmonids, specifically for the Sea trout and also for eels. The Sea trout and the White-clawed crayfish also compete in their feeding habits. In fact, the White-clawed crayfish feeds not only on plant fragments (roots, leaves, algae, seeds), but also on benthic macroinvertebrates, in particular cadisflies, small crustaceans and fish, molluscs, annelids and small shrimp. For the proper protection and management of the Crayfish, particular attention should be paid to the repopulation of salmonids for fishing purposes, which, by determining an increase in natural predators, influences the density of the populations of the species under examination. Confirming this, preliminary research carried out in the territory of Bologna led to the capture, in various circumstances, of salmonids from mixed phenotypes, probably attributable to hybrid forms of Brook trout (*Salvelinus fontinalis*) mixed with Sea trout [*Salmo (trutta)*

trutta] of varying degrees. These discoveries were interpreted as a clear symptom of inadequate management of the repopulation carried out in the watercourse, probably due to propagation material of questionable origin and poor quality.

Conservation status in the study area

The state of conservation of this species has been estimated, taking into account the entity of the populations, deduced both from the historical reconstruction of their presence and by direct censuses conducted as part of the "Water SCIs" LIFE project".

From the data collected at the various research stations on the Prato side, a quite good state of conservation was found only in some specific stretches of the Apennine watercourses monitored.

On the Bologna side, the presence of *A. pallipes* was considered rare and numerically limited. Moreover, when also taking into account the discovery of several breeding grounds of *Aphanomyces astaci* or "crayfish plague" along the brooks of Bago and Malsacco, minor tributaries of the Limentra di Treppio torrent, the conservation status of the species was deemed generally bad.

Herpetofauna

ITALIAN CRESTED NEWT

Fig. 9 – *Triturus carnifex* (Laurenti, 1768)
Ordine: URODELA Famiglia: SALAMANDRIDAE

Systematics and identification

This is the largest of the newt species present in Italy: the female can reach a length of 18 cm, while the male does not usually exceed 15 cm; however, most specimens measure between 10 and 15 cm. Based on biochemical research, the Italian crested newt is currently considered a species separate from the *Triturus cristatus*, to which it was linked until a few years ago[32]. In both sexes, the colour of the dorsal areas ranges from a blackish-grey to olive-brown and blackish-brown, with various irregularly placed darker spots on the surface; the throat area is blackish or blackish-brown with whitish dots, while the belly area ranges from yellow to reddish-orange, with numerous blackish-brown or blackish spots, each quite varied in shape, extension and position. The male, especially during the reproductive phase, has a noticeable barbed dorsal crest and there is a whitish band on each side of the tail, often tinged with blue and with pearly hues; as a rule, the female does not have a crest and, like the sub-adults, features a dorsal and supracaudal vertebral line, which varies in colour from light greenish-yellow to yellow and orangey-yellow. The larvae, which are yellowish or brown with marbling and darker spots on the top, are characterized by a tail that ends with a long filament and particularly long, thin fingers; when metamorphosis occurs, the larvae normally reach a total length of 5-8 cm, although exceptionally, they can even exceed 10 cm.

Distribution and presence in the study area

In Europe, this species is unevenly spread throughout various areas, including southern Switzerland, the Austrian Alps, eastern Hungary, the Czech Republic, Slovenia and Croatia, Bosnia-Herzegovina, Albania, Serbia, Macedonia and the north-western part of Greece[33].

In Italy, it is spread throughout most of the continental and peninsular territory, to the south up to approximately

the 39th parallel. In Tuscany, it is observed throughout the region, islands excluded, from sea level to above 1,800 m on the Apennine chain, while it is absent in Sicily and Sardinia. The collection of available data was based on bibliographical research (publications and databases provided by the Provincial Authority of Prato - Department of Protected Areas), supplemented by interviews with people living in the area and by consulting local experts working at the La Specola Museum of Natural History - University of Florence. The results of the analysis and integrated reading of the available data revealed that this species is present in the hilly area in the central and southern part of the territory of the Province of Prato.
Campaign research was conducted at every lake under examination, both along the perimeter of the lake and also in the portion adjacent to the banks, up to depths of 60-70 cm, as well as on the shores and inside the canals, ditches, waterholes and wet meadows in the immediate vicinity of the lakes.
For every area described above, direct observations were made and water samples were taken using screens, supplemented by observations made using the appropriate optical instrumentation and by listening to the calls of the amphibians for the detection of Anurians or Salientia.

Presence of possible native or alien competitors
Among the species competing with the amphibians, there are several exotic species that, because of their ecological characteristics, are a limiting factor and also a threat to the development of the biological cycle of the target species under examination. Of these, the main species are:
- The Pond slider (*Trachemys scripta*)
- The Louisiana crayfish (*Procambarus clarkii*)
- The Coypu (*Myocastor coypus*)
- The Bullfrog (*Lithobates catesbeianus*)
- The Black bullhead (*Ameiurus melas*).

Research conducted at the site of Lake Bogaia revealed the presence of one population of Pond sliders (*Trachemys scripta*) consisting of approximately 5 adult specimens and 1 young specimen (6 ind.); moreover, numerous Coypu (*Myocastor coypus*) droppings (>50 findings) were observed, leading to the conclusion that at least 2 specimens were probably present.
In the Lake Ombrone site several remains of Louisiana crayfish were found (*Procambarus clarkii*) (estimated number > 200 specimens) along with the probable dens of Coypu (*Myocastor coypus*) (estimated number approx. 2 specimens).
In the Lake Pantanelle site there were a large number of Louisiana crayfish (*Procambarus clarkii*) adult specimens and larvae in a very high concentration, up to 200 specimens /10 cl (estimated n. > 1,000 specimens); Coypu excrements were also found and the presence of the Bullfrog (*Lithobates catesbeianus*) seemed possible (and was later confirmed). There appeared to be around 5 specimens of Coypu (*Myocastor coypus*) present. The introduction of these species in the small lakes probably occurred through the minor watercourses surrounding the area under examination, which are also a potential vehicle of polluting substances and refuse.

Conservation status in the study area
Essentially, the conservation status of the T. carnifex species in the Province of Prato appears to be worsening. However, based on several findings, the presence of this species has been confirmed in some areas of natural interest (Monti della Calvana, Monteferrato) in the hilly and mountainous area of the province. In the flat part of the alluvial plain, based on the first research campaigns, the conservation status of this species is very poor, and no examples of the Italian crested newt were found at all. This negative fact, on a provincial scale, can be attributed predominantly to the poor conservation status of potentially suitable habitats for the Italian crested newt in the alluvial plain. First of all, the plain is currently too fragmented, from an environmental point of view, for the conservation of vital populations of this species. The bodies of water being investigated have also revealed

a series of significant problems (poor water quality, presence of alien species with large populations, improper management of the wet areas, shores with unsuitable slopes and invasive vegetation often out of control).
The species appears to be constantly decreasing in the territory of Prato, both from the point of view of its spreading and also from that of population density, similarly to what is happening to some extent throughout its geographical area of distribution[32].
The interviews carried out revealed that the Italian crested newt must have been present in the canals and small watercourses around Lake Pantanelle until at least fifteen years ago, though it was not present in the waters of the lake itself. As far as Lake Bogaia is concerned, in the past, the Crested newt must have been present in the various trenches and canals surrounding the little lake, but not in its waters. Lastly, the species was never detected in the area surrounding Lake Ombrone, which has a different management system than that of the other two bodies of water.

Bird fauna

Foreword

For the area of the Plain, with very little up-to-date published information (the last document dates back to 1999, by LIPU (Italian Bird Protection Society[34]), reference has been made to a specific study conducted on 37 wetland areas in 2001[35] and also to a check-list, updated in 2008[36].
In the Plain, between 1982 and 2008, a total of 219 species were recorded, 63 of which were nesting, 147 migratory, 51 wintering and 48 incidental. This wealth and diversity of species can be explained by the fact that, inside the "Plain system", there are wetlands that vary in their environmental structure and characteristics.
The direct monitoring of birds in the study area was implemented at Lakes Pantanelle, Ombrone and Bogaia, at two-week intervals, beginning in the month of February 2010. At Lake Pantanelle, as well as observation using binoculars and listening to calls, the playback method was also used, to detect Canefield Rails.
Overall, during the observation period covered by the preliminary studies (2010), 24 species were observed in the three lakes, counting for 11% of the species found in the plain between Florence and Pistoia. The order most commonly found turned out to be that of the Charadriiformes, with 8 species, followed by the Ciconiiformes with 6 species. The Birds of prey (the Accipitriforme order) were represented by one species, the Western Marsh-harrier, observed in migration near Lake Pantanelle. Lake Pantanelle turned out to have the most species (no = 17) followed by Lake Ombrone (no = 11) and by the small Lake Bogaia (no = 3). The only nesting grounds were found near Lake Pantanelle, where 29% of the species found have reproduced. In particular, the reproduction of the Coot was recorded (7 pairs), as well as that of the Great Crested Grebe (1 pair), the Little Grebe (1 pair), the Black-winged Stilt (3-4 pairs) and the Common Moorhen. In the area surrounding Lake Pantanelle a specimen of the Red-backed Shrike (*Lanius collurio*) was also spotted.
The fact that no breeding was found in Lakes Ombrone and Bogaia and that a smaller number of species was also found can be explained by the environmental conditions. Over ¾ of the surface area of Lake Ombrone had drained away completely by mid-March. The only areas still containing some water were a few puddles filled by rainwater, hosting sporadic migratory birds. Because of its size (0.45 ha) and quality of its shores (that had deteriorated, with an abundance of refuse and a sparse covering of vegetation) Lake Bogaia proved to be unsuitable for hosting nesting or migratory species.
Of the target species identified by this project, Lake Pantanelle proved to be an important feeding area for the Little Egret, of which a maximum of 10 specimens were observed during spring 2010.
Listed below, are the general characteristics of the main species of conservation interest that were found. For further information, please refer to the reports available on the project website (http://life.provincia.prato.it).

FERRUGINOUS DUCK

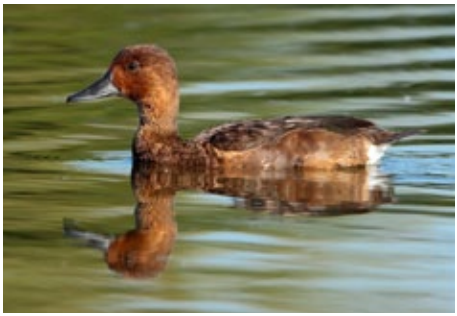


Fig. 10 – *Aythya nyroca* (Guldenstadt, 1770)
Ordine: ANSERIFORMES; Famiglia: ANATIDAE

Systematics and identification

A small diving duck. The adult male has a reddish-brown plumage with a darker back, white eye and white rump area, which is an identifying feature, distinguishing it from the female of the Tufted duck (*Aythya fuligula*). The female of the Ferruginous Duck is similar to the male, but has duller colours and a dark eye.

Distribution and presence in the study area

A monotypic species of Euroturanic phyto-geography. It nests in South-eastern Europe and Asia, with its main range reaching as far west as Poland and Hungary. Further west its presence is sporadic and irregular. In Italy, it is considered rare and has a reduced reproductive area, while it is more widespread as a migratory and wintering species. Recently, this species appears to be increasing, both from a numerical point of view and also in its area, thanks to a general improvement in environmental conditions and to greater protection. The Italian population is around 10% of that of the European Union, although it is not significant in comparison with the worldwide population of the species. The Italian population is estimated to be 78-107 pairs and on the increase[37].

In Tuscany, the nesting population is estimated to be 1-5 pairs[38], with higher numbers (up to 10 pairs) in favourable years (1990); in winter, a maximum of 27 specimens was recorded in 1992. The Ferruginous Duck has been observed in the plain between Florence and Pistoia, as irregularly migratory and rarely wintering. In the monitoring activities carried out in 2010, the species was not found, nor was it possible to ascertain its presence by interviewing hunters, as it is not a species of interest to hunters and, therefore, it is not easily recognized.

BLACK-CROWNED NIGHT HERON

Fig. 11 – *Nycticorax nycticorax* (Linnaeus, 1758)
Ordine: CICONIIFORMES; Famiglia: ARDEIDAE

Systematics and identification

The Black-crowned Night Heron is a small ardeidae; the adult has a black back and a crown with bluish tinges, in contrast to its light grey lower parts. It has short, yellowish legs. The young have a brown plumage mottled with white.

Distribution and presence in the study area

A polytypic species with sub-cosmopolitan distribution (absent from the Australasian region): the nominal subspecies lives in Europe, Asia and Africa. In Italy, the Black-crowned Night Heron is a migratory nesting and partially wintering species. Its reproductive areas are concentrated mainly in northern Italy, in the Po plain; it is less common in the rest of Italy, while it is a very localized in the south and on the islands. This species underwent a considerable increase within the European Union in the period from 1970 to 1990, followed by stability between 1990 and 2000[37]. The Italian

population is equal to approximately half of the population of the European Union and represents between one fifth and one sixth of the total European population, approximately; it probably represents the most sizeable European population.
The Italian population is estimated to be 12,000-14,000 pairs, subject to fluctuations in the period from 1990 to 2000. In Tuscany, the population increased to 610-750 pairs in the period from 1995 to 1997[38] and to 815-858 pairs distributed between 9 heronries in 1998. The Black-crowned Night Heron is reported in the plain between Florence and Pistoia as a nesting, wintering and regular migratory species. Since 2007, it has been breeding in the area of Fucecchio. It has been observed only occasionally in the study area at Lake Ombrone, while this species is present in large numbers at the nearby Lake of Querciola in Quarrata (PT). This species has not been observed at Lake Pantanelle, probably due to the lack of suitable shallow water areas in which to find food.

LITTLE EGRET

Systematics and identification

The Little Egret is an Ardeidae characterized by a completely white plumage; its beak and legs are black, while its feet are yellow. When in flight, its neck folds into an S-shape.

Fig. 12 – *Egretta garzetta* (Linnaeus, 1766)
Ordine: CICONIIFORMES; Famiglia: ARDEIDAE

Distribution and presence in the study area

A polytypic species with paleo-arctic-afrotropic-australasian distribution: the nominal subspecies nests in southern Europe, southern Asia, Northwest Africa, Cape Verde, East Africa and South Africa. In Italy, this is a nesting, migratory, partially wintering species (several thousands of specimens). The nesting areas are mainly concentrated in northern Italy; they are less common in the rest of Italy, with a scattered presence in central and southern Italy and in Sardinia. In the European Union member states, the species underwent a considerable increase in the period from 1970 to 1990, followed by more moderate growth in the period from 1990 to 2000. The Italian population represents approximately one third of the population of the European Union and approximately one fifth of the overall population in Europe. In Tuscany, Tellini Florenzano et al. (1997)[38] recorded 179-250 pairs in the period from 1982 to 1992 and, more recently, 560-720 pairs, of which 250 in the lagoon of Orbetello; the wintering groups included 300-450 specimens in the period from 1995 to 1997. Scoccianti & Tinarelli (1999)[39] reported 426-452 pairs in 10 colonies in 1998 (192 pairs at the lagoon of Orbetello). In the plain between Florence and Pistoia, the species is considered regularly migratory, nesting and regularly wintering. Inside the Cascine di Tavola, there is a winter dormitory of approximately 110 specimens, which accommodates a large part of the wintering population of the plain between Florence and Pistoia. In the study area, the species was observed at the lakes of Ombrone and Pantanelle with a maximum of 13 and 10 specimens respectively. However, the sightings at Lake Ombrone refer to the months of March and April, when a few puddles filled with rainwater were still present.

BLACK-WINGED STILT

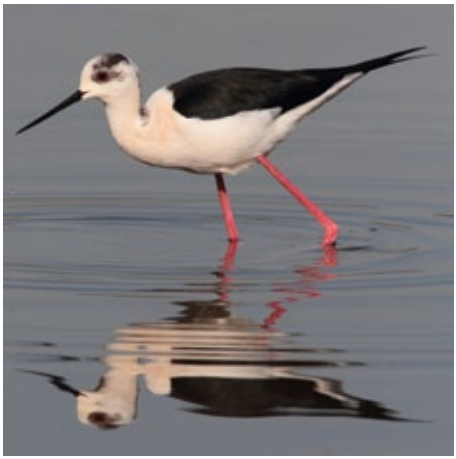


Fig. 13 – *Himantopus himantopus* (Linnaeus, 1758)
Ordine: CHARADRIIFORMES; Famiglia: RECURVIROSTRIDAE

Systematics and identification

The Black-winged Stilt can be recognized by its long, reddish legs, thin, black beak, white head, neck and underbelly and black wings and back.

Distribution and presence in the study area

A cosmopolitan polytypic species. The nominal subspecies nests in Eurasia and Africa. It is migratory, winters in Africa, mainly south of the Sahara and also locally in the Mediterranean basin, including Italy. This species showed stability within the European Union in the period from 1970 to 1990 and in the period from 1990 and 2000[37]. The Italian population consists of approximately 3,000-4,000 pairs, which increased in the period from 1990 to 2000 and corresponds to approximately 13%-15% of that of the European Union. In Tuscany, the population fluctuates between 20 and 200 pairs, with a net decrease from the mid-1980s due to the drastic fall in the populations at Massaciuccoli and Orbetello, which was only partially balanced by the increases at Diaccia Botrona and in the inland lakes[38]. In the plain between Florence and Pistoia, the species it is considered regularly migratory and nesting. The population fluctuates in relation to the varying levels of floodwater during the reproductive period. At Lake Ombrone, the species was observed near the few pools of water which were in the process of drying up, while at Lake Pantanelle there were 3-4 breeding pairs.

KINGFISHER

Fig. 14 – *Alcedo atthis* (Linnaeus, 1758)
Ordine: CORACIIFORMES; Famiglia: ALCEDINIDAE

Systematics and identification

The Kingfisher has an unmistakable bright blue plumage, with metallic hues in the top areas, orange on the belly, abdomen and cheeks, white on the throat and on the sides of the neck. The young have the same colouring as the adults. Both sexes are similar.

Distribution and presence in the study area

Polytypic species with paleo-arctic-eastern phytogeography. The nominal subspecies lives in Northwest Africa, southern and eastern Spain, Corsica, central and southern Italy, south-eastern Europe, Turkey, the Middle East, extending eastwards as far as the northwest of China; the subspecies *Alcedo atthis ispida* instead occupies Europe to the north and to the west of the nominal subspecies. In Italy, it is a stationary, migratory and wintering species. The European populations suffered a significant decline in Europe in the period from 1970 to 1990, while they were stable in the period

from 1990 to 2000[37]. The Italian population was estimated at 5,000-10,000 pairs in 2000 and is between 15% and 18% of the population of European Union (8%-10% of the overall European population). In the Plain, it is considered a sedentary, nesting, regular migratory and scarcely wintering species. Lake Pantanelle is frequented by this species for trophic reasons, but it does not nest here due to the lack of suitable habitats.

RED-BACKED SHRIKE

Systematics and identification

The adult male has a grey crown and nape, a tawny-brown back and inner part of the wings and blackish-brown ends of the wings. It has a black beak, legs and mask. The cheeks and the throat are white. The rump is grey and the tail is black with two white lateral bands. It has pink under-parts. The female adult is similar to the male, but differs because of her crown, which is a fairly light brown colour, fading to grey near the nape, a mask that is pale at the front and brown behind the eye and her lower areas are off-white with dense grey-brown mottling. The young are mostly brown on the back with some off-white mottling.

Fig. 15 – *Lanius collurio* (Linnaeus, 1758)
Ordine: PASSERIFORMES; Famiglia: LANIIDAE

Distribution and presence in the study area

A polytypic species with Eurasian distribution; migratory nesting, winters in Africa. Europe is home to less of the half of the total population. In Italy, the Red-backed Shrike is relatively common, from the coastal areas to the mountains, up to altitudes of almost 2,000 m. The species underwent a considerable decline in most of the European area in the second half of the twentieth century and a moderate decline in Europe in the period from 1970 to 1990, while the general population of the continent remained stable or underwent a light decline in the period from 1990 to 2000[37]. The Italian population is estimated at 50,000-120,000 pairs; in slight decline (<20%) in the period from 1990 to 2000. The Italian population is between 2% and 8% of the population of the European Union and represents approximately 1-2% of the total European population. In Tuscany, there were an estimated 5,000-20,000 pairs during the 1990s, probably decreasing[38] and undergoing a clear decline in the decades that followed. In the Plain, the species is considered regularly migratory and nesting. In the study area the species was spotted in an uncultivated area near Lake Pantanelle.

As well as the six species described above, monitoring activities performed during the preliminary studies revealed the presence of numerous other bird species of conservation interest, of which, for the sake of brevity, we have only described those whose presence in the area was confirmed by subsequent wildlife censuses carried out during the course of the project.

SQUACCO HERON

Fig. 16 – *Ardeola ralloides* (Scopoli, 1769)
Ordine: CICONIIFORMES; Famiglia: ARDEIDAE

Systematics and identification

The Squacco heron has a predominantly tawny-yellow colour on its back and upper parts, while the top part of the wings, from the body outwards, blends immediately into a very light tawny colour, which is nearly white at the extremities (in flight, it looks very light on top). On its crown, it is a beige colour, streaked with brown.

Distribution and presence in the study area

A monotypic species with paleo-arctic-afrotropic distribution. In Europe, it is common mostly in the south, south-east. In Italy, the species is migratory nesting and rarely wintering, since it winters in Africa. The breeding areas are concentrated mainly in northern Italy; it is less common in the rest of Italy, with a scattered presence in the centre, in Puglia and on the islands. This species underwent a moderate decline within the European Union in the period from 1970 to 1990, followed by stability between 1990 and 2000[37]. The Italian population is about 22%-25% of the population of the European Union and represents approximately 2-4% of the overall European population. The Italian population was estimated at 550-650 pairs in the period from 1990 to 2000.
In Tuscany, the species was reported as a nesting species in 1998, with 27-38 pairs in 4 colonies[39]; in Padule di Fucecchio, the species was reported as a nesting species with 2-5 pairs in 1984-85 and 16-58 pairs between 1998 and 2000[40].
This species is reported in the plain between Florence and Pistoia as scarcely migratory, nesting and occasionally wintering. In the study area, it has been spotted during spring migration at Lake Pantanelle.

GREAT EGRET

Fig. 17 – *Casmerodius albus* (Linnaeus, 1758)
Ordine: CICONIIFORMES; Famiglia: ARDEIDAE

Systematics and identification

This is a large egret, characterized by a completely white plumage; it differs from the Little Egret not only because of its larger size, but also for its yellow beak and light-coloured feet.

Distribution and presence in the study area

A cosmopolitan polytypic species: the nominal subspecies nests in Europe and in the temperate part of Asia. Its distribution in Europe is fragmented and discontinuous. The main populations are found in eastern and south-eastern countries. In Italy, this species began breeding in 1990 in the Po Delta, and has since been expanding; before this, it was only reported as a migratory and winter-

ing species. This species underwent a moderate increase within the European Union in the period from 1970 to 1990, followed by a considerable increase between 1990 and 2000[37]. The Italian population is approximately 1.1%-1.5% of the population of the European Union and represents a non-significant fraction of the overall European population. In the plain between Florence and Pistoia, it is considered regularly migratory and rarely wintering. In the study area, several specimens of this species have been found in Lakes Pantanelle and Ombrone.

WHITE STORK

Systematics and identification

This species is unmistakable due to its large size and its plumage, which is white, except for the black flight feathers, and its very noticeable, bright orange feet and beak.

Fig. 18 – *Ciconia ciconia* (Linnaeus, 1758)
Ordine: CICONIIFORMES; Famiglia: CICONIIDAE

Distribution and presence in the study area

A polytypic species with European-Central Asian-Mediterranean phytogeography. In Europe, it is present mainly in countries to the east and in the Iberian region. The White stork had been extinct in Italy since the late Middle Ages, but started to nest in our country again in 1959, in Piedmont, spreading to other regions in the decades that followed, also thanks to widespread reintroduction activities. In Italy, it is predominantly a trans-Saharan migratory species, although there have been increasingly frequent sightings of wintering specimens. This species underwent an extensive decline within the European Union in the period from 1970 to 1990, followed by a considerable increase between 1990 and 2000[37]. The Italian population amounted to 103 pairs in 2002, equal to approximately 0.1% of the population of the EU. Initially (in 2010) it was not found in the area, but since 2011 a pair has been breeding continuously in the area of study (Iolo), in the immediate vicinity of Lake Ombrone, and was repeatedly spotted flying over Pantanelle during the spring and summer months.

WESTERN MARSH-HARRIER

Systematics and identification

With a classic silhouette, typical of the harrier, the Western Marsh-harrier differs from this species due to its plumage, which is dark brown on top, with a slate grey tail, in contrast with its grey secondary flight feathers and black primary flight feathers and a creamy-brown head, neck and chest, with streaks in the male of the species. The female is brown with a cream-coloured nape, throat area and shoulders.

Fig. 19 – *Circus aeruginosus* (Linnaeus, 1758)
Ordine: FALCONIFORMES; Famiglia: ACCIPITRIDAE

Distribution and presence in the study area

A polytypic species with a paleo-arctic-Afrotropic-australasian phytogeography. In Italy, this is a station-

ary nesting species, but it is also migratory and wintering. The distribution of the nesting pairs is very irregular and localized. This species underwent a moderate increase within the European Union in the period from 1970 to 1990 and in the period from 1990 to 2000[37]. The Italian population was estimated at 170-220 pairs, in moderate increase in the period from 1990 to 2000. Italy is home to a nesting population that is less than 1% of that of the European Union and is not particularly significant on a pan-European scale. In Tuscany, the number of breeding pairs has increased from 16-18 in the 1980s to 33-37 in 2002-2004. In the plain between Florence and Pistoia, the species is considered irregularly wintering and regularly migratory; it has been spotted near Lakes Pantanelle and Ombrone during spring migration (both male and female specimens).

RUFF

Systematics and identification

The adult males are considerably larger than the females and have a particularly showy breeding plumage with a collar that stands up during fights and display rituals between males. Colouring varies considerably from bird to bird, ranging from white to red and grey, etc. The back, the rump and the upper part of the wings are quite constant and are brown in colour, with little black markings and lighter borders around the feathers. In spring, the colouring of the females is similar to that of the males, with the same plumage but without the different-coloured tufts of the male's breeding plumage. With their winter plumage, the upper parts and the chest of both males and females are predominantly grey. The under-parts (abdomen and belly) are whitish.

Fig. 20 – *Philomachus pugnax* (Linnaeus, 1758)
Ordine: CHARADRIFORMES; Famiglia: SCOLOPACIDAE

Distribution and presence in the study area

A species with Euro-Siberian distribution. In Europe, the Ruff nests in the eastern and northern regions, occupying large areas in Russia and Scandinavia and becoming gradually less common, moving towards the south and the west. In Italy, its presence is massive during migrations and, secondly, during the winter season. Several hundred specimens spend the winter in the inland and coastal wetland areas of the Northern Adriatic Sea and of central Italy, Puglia, Sicily and Sardinia. In the Plain, the Ruff is considered regularly migratory. In the study area, it is the most numerous species during migrations: 60 specimens were spotted simultaneously at Lake Ombrone and 40 specimens at Pantanelle.

WOOD SANDPIPER

Fig. 21 – *Tringa glareola* (Linnaeus, 1758)
Ordine: CHARADRIFORMES; Famiglia: SCOLOPACIDAE

Systematics and identification

The male and the female have a virtually identical plumage with a brown colouring, dotted with light markings in their upper parts and off-white in the under-parts.

Distribution and presence in the study area

A monotypic species with Euro-Siberian distribution. The Wood Sandpiper is common in the northern regions of Europe, roughly from the 50th parallel to the Arctic coasts, including Scandinavia, Russia and the neighbouring regions. It winters in tropical and subtropical areas in Africa and Asia. In Italy, its presence is massive during migrations and it is exceptionally present as a wintering bird. In the Plain, it is considered regularly migratory and irregularly summering. Within the study area, it was only been spotted near Lake Pantanelle (31 contacts) and Lake Ombrone (14 sightings).

PURPLE HERON

Fig. 22 – *Ardea purpurea* (Linnaeus, 1766)
Ordine: CICONIIFORMES; Famiglia: ARDEIDAE

Systematics and identification

This species belongs to the family of the ardeidae and is considerably large. Its wingspan can reach 1.50 m; it is 78-90 cm in length and can weigh up to 1.4 kg. It is slightly smaller than the White heron. It has a long, reddish-brown neck (from which it gets its specific name) in an S-shape with a distinctive black stripe; the top of its head is black. It has brown markings on its wings. During the courtship period, it acquires a much more attractive plumage, especially on the neck.

Distribution and presence in the study area

The range of distribution of the Purple heron includes, in particular, the south-western paleo-arctic area and, to the East, it reaches Turkestan and Iran. It also nests in East Africa and South Africa.
It is a long-range migratory species that winters mainly in sub-Saharan Africa to the north of the Equator[41]. In Italy in the 1970s, the population of this species fell sharply[42]. Its preferred habitat is the canefield and wet shrubby formations in general. In Tuscany, this is a migratory and nesting species.
The Purple heron was spotted at Lake Pantanelle, where a young specimen was also found, making the idea of nesting locally a plausible one.

LITTLE BITTERN

Fig. 23 – *Ixobrychus minutus* (Linnaeus, 1766)
Ordine: CICONIIFORMES; Famiglia: ARDEIDAE

Systematics and identification

The Little Bittern belongs to the heron family, but it differs from the other ardeidae due to its small size: 33-38 cm with a wing span of 52-58 cm. It has dark wings, the underside of which are a cream colour. In the male, the top part of the head and the back are black with greenish tinges, while the female features a more tawny colouring in her underbelly and dark stripes over the back. The beak is yellow-green, the eyes are yellow and the legs are greenish.

Distribution and presence in the study area

A species with sub-cosmopolitan distribution. This is a summer migratory species that is stationary in Tuscany from April to September. A great migratory species, it winters in sub-Saharan Africa. Our country, where the Little Bittern is a migratory and nesting species, is crossed by a substantial migratory flow of populations from Central and Central-Eastern Europe[41].
In the area involved in the project, this target species was detected in the Pantanelle wetlands, both as a result of its call and by direct sightings. The call of the species allows us to hypothesize a small probability of nesting in the area. It prefers riparian vegetation, especially phragmites.

EURASIAN SPOONBILL

Fig. 24 – *Platalea leucorodia* (Linnaeus, 1758)
Ordine: CICONIIFORMES; Famiglia: THRESKIORNITHIDAE

Systematics and identification

The Eurasian Spoonbill is a very large, heavy bird: it can measure up to 85 cm in height and can weigh as much as 2 kg. Its most noticeable feature is its spatula-shaped beak, from which it gets its name.
The Eurasian Spoonbill's plumage varies based on the season: in winter it is completely white, while in the breeding season several yellowish markings appear, mainly at the base of the neck and on the nape.

Distribution and presence in the study area

The species has a fragmented breeding area in Europe, limited to a few sites along the Atlantic coast (France, Holland), in Mediterranean countries and in the Balkans, where it occupies both internal and lagoon wetland areas[41].
In Italy, these birds have been found to winter in various regions (Sicily, Tuscany, Puglia), while 90% of the nesting specimens are situated in the Po Delta and some also winter along the Tyrrhenian coast (Orbetello or Burano).
In Tuscany, the Eurasian Spoonbill is a migratory and wintering species which lives in shallow ponds with hygrophilous vegetation.

The several specimens of the species have been spotted at Lake Ombrone and also at Lake Pantanelle.

The presence of possible native or alien competitors

In the lakes of the Plain, the only alien species that may have an impact on the bird fauna is the Coypu, since there were no sightings of the American mink (*Neovison vison*[43]), the other potential competitor. On the other hand, the Louisiana crayfish (*Procambarus clarkii*) is used as a food source, particularly by the ardeidae.

The Coypu can damage nesting bird populations both indirectly (by feeding on various species of aquatic plants, like *Typha angustifolia*, *Typha latifolia*, *Nymphaea alba* and *Trapa natans*) and directly, by overturning or sinking nests built on aquatic plants, which are either partially submerged or positioned on the edges of the canefields, as a result of the occasional predation of eggs. It can also cause damage to the banks of the wetlands, caused by the activity of digging burrows.

In the Valleys of Argenta, in Emilia Romagna, an increase in the number of Coypu coincided with a sudden decrease in the populations of the Great Crested Grebe (*Podiceps cristatus*), the Little Grebe (*Tachybaptus ruficollis*) and the Whiskered Tern (*Chilidonias hybridise*)[44].

Among the species nesting in Lake Pantanelle, the Coypu could cause harm to the Great Crested Grebe, the Little Grebe, the Coot and the Black-winged Stilt (one of the project's target species).

The native species that may represent an element of competition to the bird fauna, as far as the population spread is concerned, include mammals like the Fox (*Vulpes vulpes*) and birds like the Yellow-legged Gull (*Larus michahellis*) and the Grey crow (*Corvus corone corone*), potential predators of the aquatic birds' eggs or pullets. During the research, no fox excrements were found, while a specimen of the Yellow-legged Gull was spotted at Lake Ombrone. The species was also seen at Lake Caserane and "La Querciola" in Quarrata. Although the species may occasionally take to the predation of pullets and eggs, as confirmed, for example, by the territorial reaction of the Black-winged Stilt when it appeared, the number of specimens to be found during the breeding period does not represent an urgent threat to the nesting aquatic species. The same applies to the Grey crow.

At least 3-4 specimens of the Coypu are present at Lake Pantanelle. Several traces were also found at Lakes Bogaia and Ombrone. Based on a qualitative estimate, the presence of species seems to be relatively low in density, in contrast to the situation at Padule di Fucecchio, where there are signs of a much more numerous presence and campaigns are under way to contain the species through capture and culling[45].

Conservation status of birdlife in the study area

The field investigations undertaken made it possible to paint a picture of the conservation status of those species that frequent Lakes Pantanelle, Ombrone and Bogaia, and also of the ecological conditions of these three artificial basins.

At the date of delivery of the preliminary studies (June 2010), Lake Pantanelle (6.2 ha) was the hunting lake with the best conservation potential and the one that hosts the largest number of species, both in general and nesting species. The relatively low number of both migratory and nesting species that frequent the area can be attributed to poor environmental diversity.

For example, the absence of any sightings of the Black-crowned Night Heron, one of the target species identified by the project, together with the low number of migratory shorebird species, can probably be attributed to the lack of suitable expanses of flooded grasslands and muddy areas of shallow water, where this species could stop off in search of food.

The canefield strip that occupies one side of the lake is also limited and needs to be expanded in order to maximize the presence of the border between the canefield and the free waters, which is a favourable nesting area for species like the Little Bittern (*Ixobrychus minutus*) and the Great Reed Warbler (*Acrocephalus arundinaceus*) and for the feeding of other ardeidae; sufficiently deep strips of canefield also make it possible to considerably reduce human disruption, as well as offering breeding sites for species like the Little Grebe, the Great Crested Grebe, the Coot and the Common Moorhen. The area is managed by hunters at the lakes, with whom a constructive dialogue was established.

Lake Ombrone is also a vast hunting lake (10.5 ha), the management of which makes it unsuitable for hosting birdlife during the migratory and breeding periods. In fact, this basin starts drying up as early as March, with residual puddles of water near small hollows that will dry up in the months that follow, due to evaporation and/or absorption by the land. Once it has dried up, the bed undergoes milling and ploughing until it appears, in late spring, as an expanse of dry mud, which is totally inhospitable for the bird fauna to stop over or breed. The banks are also completely lacking in riparian vegetation and canefields and there is virtually no diversification in the level of the waters. In contrast to Lake Pantanelle, the hunters who manage this lake basin have not shown any readiness or willingness to adopt management methods that are more oriented towards the conservation of the nature and biodiversity in the area.
Lastly, Lake Bogaia is a decidedly small body of water compared to the other two (0.45 ha). The wetland area comprises various critical conditions for the bird population, including its small size and the quality of the waters and of the shores. The adjoining agricultural area on the other hand is frequented by ardeidae: as many as 11 specimens of Cattle Egret (*Bulbucus ibis*) were spotted on a single occasion.

In-depth study of invasive alien species in the Prato plain

The wetlands that remain in the highly urbanised ecological mosaic that characterizes the plain of the metropolitan area of Florence, Prato and Pistoia are still continuously subjected to activities that pose a significant threat and to elements of pressure, not least of which is the presence of invasive alien species. These are numerous both in the quantity of species present and also in the abundance of their relative populations.
The impact that these species has on biodiversity represents the second threat to its conservation, exceeded only by the destruction/alteration of the habitats[46]. As a result, the European Commission has included tackling invasive alien species among the core elements of the EU Biodiversity Strategy (Communication COM 2011/244).
In view of this, during the project we decided to initiate a specific activity of analysis, monitoring and experimentation of possible methods for controlling the populations of invasive non-native species present in the areas involved in the requalification measures, including those illustrated below. These activities were carried out by Nemo S.r.l., a company selected through a public tender, with a work group including experts from the University of Florence.
Screening carried out in 2013 allowed us to confirm the presence, already highlighted during preliminary studies, of numerous invasive alien species in the Prato area of the “Ponds of the Florentine and Prato plain” SPA, among which there were numerous species of fish (e.g. Wels catfish, Stone moroko, Common catfish, Grass carp, Pumpkinseed sunfish, Gambusia and Common bleak) and other species common to damp environments (e.g. Louisiana crayfish, Bullfrog, Pond Slider and Coypu). Among the invasive species of alien flora in the part of the SPA in question, we also confirmed the presence of the Bohemian knotweed, the Ailanthus and the Black locust. The following pages only describe the species which we considered to pose the greatest threat to the target species, based on the size of the populations observed and their invasive potential described in literature, and against which we decided it was necessary to intervene, by experimenting containment methods that will be covered in greater depth in the chapter dedicated to concrete conservation measures.

Astacofauna

LOUISIANA CRAYFISH

Fig. 25 – *Procambarus clarkii* (Girard, 1852)
Ordine: DECAPODA; Famiglia: CAMBARIDAE

Systematics and identification

The Louisiana crayfish, or Killer crayfish, is a freshwater decapod native to North America. The adult specimens have a medium length of approximately 15 cm, although they can also reach 20 cm. They have a distinctive dark red or reddish-brown colouring, making it easy to distinguish them from the native species; blue, yellow, black and white varieties are also known. The cephalothorax (the front part of the body, comprising the head and the thorax fused together, where the legs are found) is rough, and extends forward into a pointed, narrow rostrum that gradually broadens from apex to base. The pincers are well-developed and bumpy, larger in size in the male specimens and covered with barbs and tubercles.

The Louisiana crayfish has a series of features that make it an excellent invader. As far as feeding is concerned, it is a generalist and opportunist species[47], with adults that eat mainly plants and vegetable detritus, while the diet of the young includes a larger amount of animal protein.

In a natural environment, its biological cycle does not generally exceed 12-18 months[28]; for a crayfish, it therefore has a relatively short lifespan, although it is known for its rapid growth, early maturity and high fertility[48]. In fact, it reaches sexual maturity at total length of 45 mm and the females can produce up to 600 eggs, with the number of eggs increasing according to the size of the female. There are no larval phases and its development is direct. The females keep their eggs (and then the young, which look like the adults) in the ventral part of the abdomen. Embryonic development and growth are both temperature-dependent and come to a halt below 10°C. Growth occurs by shedding, when the Crayfish abandons its "old shell" in order to increase its size and create a new one. In this phase, the Crayfish is particularly vulnerable to predators and to conspecifics and takes shelter in its cave.

The preferred habitats of the Louisiana crayfish are lentic environments, like swamps and marshes, including those with strong seasonal water level fluctuations, even to the point of temporarily drying out; *P. clarkii* is, however, able to colonize any type of aquatic environment, including brackish waters. In urban and peri-urban areas, it has no difficulty colonizing canals and gulleys.

It actively digs burrows in the banks, where it passes the winter and the more delicate moments during its life cycle (post-shedding phase, reproduction). These burrows range from 50 cm in depth for temporary ones to up to 5 m for permanent nests. They have stagnant water on the bottom and may be simple tunnels or have an entrance way formed of plugs of mud. This burrowing activity is particularly intense in silt-clay soils. These animals are not particularly faithful to their burrows.

The species is able to tolerate extreme environmental conditions, including chemical pollution, high temperatures and drought[49]. Its resistance to parasites and diseases is documented, in particular to the fungus Aphanomyces astaci, the so-called "crayfish plague", of which *P. clarkii* is a healthy carrier[50]. This disease is the main culprit for the blight of native crayfish in the last years.

Distribution

The species originated in the central and southern areas of the United States and north-eastern Mexico. It was introduced mainly for reasons of aquaculture in every continent, with the exception of Australia and Antarctica. In Europe, it was introduced for the first time in Spain, in 1973[51] and its presence is still certain today in 13 countries, including Italy.
In Italy, the presence of *P. clarkii* was first reported in 1989 in the Banna torrent in Piedmont, and subsequently, in 1993, in Lake Massaciuccoli in Tuscany, where it was introduced for aquaculture. In this last case, its spread into nature was caused by several specimens escaping from the breeding tanks. The species is common in most of central and northern Italy and has recently also been reported in Sardinia, Sicily and several areas in the south. In Tuscany, it is present in all provinces in the region, with particularly abundant and invasive populations in the plain between Florence, Prato and Pistoia, and in the wetland areas of Tuscany (Fucecchio, Massaciuccoli). In the lakes of Pantanelle, Bogaia and Ombrone, sites that are subject to intervention in the "Water SCIs" LIFE project, *P. clarkii* is very abundant. The presence of the species has also been confirmed in the canals around Lakes Pantanelle and Ombrone.

Impact

This species has been included in the list of the 100 worst invasive species in Europe[52]. Thanks to the characteristics that make it a perfect invader of the aquatic ecosystems, and to its high population density, the loss of biodiversity caused by its presence has been widely documented. In fact, it causes the local extinction of numerous species of macro-invertebrates (particularly the native European crayfish, to which it is able transmit the crayfish plague), fish and amphibians[53]. It also transmits the *Batrachochytrium dendrobatidis* fungus, which is the most significant threat for Amphibians worldwide. When considering its ability to tolerate and to accumulate within its tissues heavy metals, microalgal[54] toxins and other bacteria that are pathogenic for man[55], this species is potentially harmful for the health of anyone consuming its flesh, should it come from non-controlled conditions. The intense burrowing activity of *P. clarkii* causes structural damage to artificial embankments[56] and induces bioturbation of the water, with a consequent reduction in primary productivity.

Herpetofauna

BULLFROG

Systematics and identification

It is 20 cm long including the head and body and weighs over 1.5 kg, making it the largest species of anura in Italy. Its dorsal area varies in colour, from bright green to dark brown, with variable dark markings; the belly is whitish, or sometimes mottled grey. It has very large eardrums, similar in size to the eye: in the male, these are larger, and can be as large as double the diameter of the eye. The larvae, which generally reach a length of 10 to 13 cm at the end of their development phase, have olive green or dark olive-brown dorsal areas with blackish markings and their lower parts are whitish or yellow.
The Bullfrog can be found in open, fairly deep bodies

Fig. 26 – *Lithobates catesbeianus* (Shaw 1802)
Ordine: ANURA; Famiglia: RANIDAE

of water, like small lakes, swamps, waterholes and ponds of medium-large sizes, torrents, slow-flowing canals, etc. The male produces a powerful and distinctive croak that sounds like a bovine lowing noise, from which the species gets its common name. The female is, however, also able to emit a series of distinctive croaks.
The female chooses a male and lays her eggs in his territory: during the release of the eggs, the male fertilizes them, simultaneously pouring his spermatozoa over them. Breeding is usually in late spring and every female lays up to 20,000 eggs, arranged in a wide, thin layer (diameter 50-150 cm) on the surface of the water. The larvae generally require 2-3 years or, more rarely, only one year to reach metamorphosis and they develop at temperatures between 24 and 30°C. Those who have gone through metamorphosis spend winter in the mud on the bottom of a body of water, in cavities on the banks or even on the ground, under various types of shelter.

Distribution
The Bullfrog originated in North America, east of the Rocky Mountain chain. It was introduced, mainly for food purposes, in at least 40 States and 4 continents. Today, its presence is reported in the United States as well as west of the Rocky Mountains, in Canada, in the Islands of Bermuda, in most of Central and South America and in the Islands of the Caribbean, in the islands of Hawaii, in South-eastern Asia, in Japan and in numerous countries of Western Europe (Great Britain, where it has been eradicated, Belgium, Holland, France, Spain, Germany, Switzerland, Italy, Greece).
In Italy, the species was first imported for culinary purposes into the waters of Corte Brusca (Mantua) between 1932 and 1937 and, from here, by natural expansion or successive introductions, it spread through most of the Po plain[57], giving rise to the current populations, which started from a very small number of founding specimens. Also the populations present in Lazio (south-western area in the province of Rome) come from specimens imported in 1974, seemingly with fish repopulations, from Castel d'Ario (Mantua). The species then spread throughout the whole of central and northern Italy, also because of initiatives aimed at its breeding for food purposes.
The Tuscan population comes from a few young specimens from the United States, which were introduced into the waters of the Fosso Vermiglia (Quarrata, Pistoia) in the early 1970s. Between 1976 and 1977 the presence of the Bullfrog was been detected in the Stella torrent (Quarrata), the Fosso Tozzinga (Bisenzio Fields, Florence) and near Comeana (Carmignano, Prato), all localities in the basin of the Ombrone Pistoiese torrent[58]. At the end of the 1970s, there was an abundant presence of adult, larvae and young Bullfrogs in the areas surrounding La Catena (Poggio a Caiano-Quarrata) and Quarrata[59]. The species was then reported to be present in the ponds of Castelletti (Signa, Florence), Castelnuovo (Prato), Padule dell'Osmannoro (Florence) and at the Tenuta di San Rossore (Pisa)[57]. It is present in the lakes of the Nature Reserve of Local Interest, La Querciola (Quarrata, Pistoia). The species is found in areas with altitudes of between 35 and 50 m. After an initial period of rapid, almost explosive territorial expansion, the Bullfrog apparently became stable or even in local decline in Tuscany, from 1985, a year in which the minimum temperatures in winter were considerably low, probably decimating the specimens in their wintering locations.
As far as the sites that are subject to intervention in the "Water SCIs" LIFE project, the Bullfrog appears not to be present in Lake Bogaia, while its presence has definitely been reported in the park of Cascine di Tavola: therefore, possible upcoming recolonization can be expected in the lake, where, until a few years ago, the species was undoubtedly present (the recent reworking of the lake may have momentarily driven many amphibians away from these waters). Moreover, the introduction of alien fish for recreational fishing has meant that these fishes have decimated the amphibians residing in the lake. The call of this species was heard at Lake Ombrone, during investigations in the summer of 2013, and also in the nearby detention basin named "Lavacchione". At Lake Pantanelle, on the other hand, during monitoring carried out the same summer, the call of the Bullfrog was heard several times and two male specimens were captured in the fish traps used form monitoring crayfish.

Impact

The species is included in the list of the 100 worst invasive species in the world and in the DAISIE list of the 100 worst invasive species in Europe[52].

This species Is very active and particularly voracious. Because of their remarkable size, the adults prey on numerous invertebrates and also on small and medium sized vertebrates, including fish, other amphibians (among which frogs, newts and the young of their own species), snakes, small marsh turtles, the chicks of aquatic birds and micro-mammals. Their impact on other species is, therefore, considerable; in particular, as far as green frogs are concerned (genus Pelophylax), the Bullfrog represents both a strong competitor and a frightening potential predator. However, a direct assessment of the impact on other amphibians in Italy is missing[60], although there is substantial evidence regarding the impact of species from many other parts of the world[61].

The Bullfrog is a healthy carrier of, or at the least scarcely affected by, the *Batrachochytrium dendrobatidis* fungus[62], which currently represents the most significant threat for the survival of amphibians worldwide: in fact, the fungus has already led to the extinction of numerous of species and has determined a shrinkage of the area occupied by other species.

The adults do not generally have any effective predators (even though there are numerous occasional predators, especially among the ardeidae), with the possible exception of rats, which can prey on them in their wintering places on the ground. This amphibian is also very tolerant to pollution compared to the native species of frogs, toads, newts and salamanders.

Many studies describe the Bullfrog as an “ecosystems engineer”, meaning that it is able to alter the biomass, structure and composition of communities.

POND SLIDER

Fig. 27 – *Trachemys scripta* (Schoepff, 1792)
Ordine: TESTUDINES; Famiglia: EMYDIDAE

Systematics and identification

The Pond slider is a medium-large sized turtle, with a shell that can reach 30 cm in length (medium range 13-20 cm). The shell is olive-brown, olive-grey or brownish-grey in colour, with more or less evident black and yellow markings. In nature, this colouring often appears a uniform greyish-brown or light greyish colour, due to the veil of mud covering the shell. Instead, the plastron is yellowish or pale yellow with dark spots which, in young specimens, are bordered by green and light yellow. There are narrow yellowish lines on the neck and the head; from behind the eye to the end of the head, there is a striking long, red marking (*T. s. elegans*) or an orangey-yellow marking (e.g. *T. s. scripta* and *T. s. troostii* and hybrid specimens[63]). In nature, when observing through binoculars, it is not always possible to see these differences in colour, especially in the bigger specimens, which tend to have a duller colouring.

The species exhibits marked sexual dimorphism[64]: the males can be easily distinguished from the females, when being handled, because they have a longer pre-cloacal tract (distance between the cloaca and the posterior lobe of the ventral plastron) of the tail and, therefore, a more external cloacal opening, outside of the rim of the shell, and a longer tail. The males generally have very long claws on their front legs, which they use in courtship rituals. They also usually have a longer snout and large adult specimens may show melanism.

The species is found, especially outside of the natural area it occupies, both in natural and semi-natural environments, like rivers, lakes, torrents, canals and waterholes, as well as in areas heavily affected by human activity

and fountains in urban environments, often in mediocre or poor quality waters.
Pond sliders are active in temperatures between 10° and 37°C. During the cold season, from mid-autumn to the end of the winter, they hibernate under the mud, as does the native *Emys orbicularis*. When the water is at an optimal temperature, the turtles are visible in the water and on top of semi-submerged tree trunks, stones and on the shores, intently basking, or warming themselves in the sun for reasons of thermoregulation. In their native territory, they mate in the water, usually in spring and in autumn and lay their eggs in late April and mid-July[65]. Each female lays 10-30 eggs on the land adjacent the banks of the body of water. Incubation lasts from 2 to 4 and a half months. The species is mostly active during the daytime. The young feed on invertebrates, small fish, larvae and juvenile amphibians, while the adults are predominantly herbivores, but also opportunist carnivores. Wintering, between mid-autumn and the end of winter, usually occurs in the muddy beds of the body of water.

Distribution
The species is native to a large area in central and south-eastern America. Because of its common use as an ornamental animal (and also for food consumption), it was subsequently introduced (especially its subspecies *T. s. elegans*) in numerous countries of the world, in particular in the rest of the United States, a large part of Asia and of Europe and also in Africa and Oceania. However, there is little evidence of breeding populations, found only in France, Spain and Italy[66,67,68].
The release into nature of specimens of this species is favoured by its longevity in captivity (up to 50 years) and by the considerable size it reaches (over 30 cm in length of the shell), factors that make it difficult to keep as a pet in private homes. The consequent continuous release of species into nature has given rise to many alien populations, in some cases with high density. In Italy, the first reports date back to the 1970s in Abruzzo, but it was not until the following decade that the species was reported relatively frequently. Currently, the area it occupies seems to be in constant expansion: the species is present in all the regions of Italy, including the islands, with the exception of Valle d'Aosta and Campania, rarely in nuclei but able to reproduce. It is found in areas with altitudes that vary from sea level to 1,500 m, although in Italy it very rarely exceeds 650 m.
In Tuscany, wild Pond sliders have been spotted in many localities, but there is no recent data regarding actual distribution and quantity. In any case, the species appears to be in constant expansion, both regarding the territory colonized and also in terms of abundance of specimens[69]. In the regional territory, the species has been spotted from sea level up to at least 385 m, in the province of Florence[69]. It is very common in urban fountains and artificial ponds, in parks and also in the urban sections of the major rivers that cross the plain of Florence, Prato and Pistoia.
As far as the areas that are subject to intervention in the "Water SCIs" LIFE project are concerned, the Pond slider has been spotted in large numbers at Lake Bogaia at Cascine di Tavola, while it appears not to be present at Lake Pantanelle and Lake Ombrone, as no animals were spotted during the monitoring activities performed. In the area of the Plain, there is no reliable indication of any cases of reproduction to date.
It is useful to keep in mind the fact that, in the Plain, the presence of the native *Emys orbicularis* has been reported, a species that may also be present in the areas in question: the most recent discoveries from nearby areas date back to 1996, in Campi Bisenzio, and to 2000, in Signa.

Impact
The species is included in the list of the 100 worst invasive species in the world and in the DAISIE list of the 100 worst invasive species in Europe[52].
In sites of cohabitation, the Pond slider can cause a strong impact on the native European pond turtle *Emys orbicularis*, a species which is already under serious threat from the reduction and degradation of the wetland areas and by anthropic disturbance. In fact, *T. scripta* competes for the same territory and food resources as *E. orbicularis*[70]. In the high density populations found in France, *T. scripta* also been observed to have a consider-

able impact on amphibians, arthropods, molluscs and aquatic vegetation[71]. The species is a carrier of several serotypes of Salmonella enterica, which can also be transmitted to humans.

Invasive alien flora

BOHEMIAN KNOTWEED

Fig. 28 – *Reynoutria x bohemica* (Chrtek & Chrtková)
Ordine: POLYGONALES; Famiglia: POLYGONACEAE

Biology of the species and local distribution

The Bohemian knotweed is a plant pertaining to the family of the Polygonaceae, which originated through hybridization between two species from East Asia: *Reynoutria japonica* Houtt. *var. japonica* and *R. sachalinensis* (F. Schmidt) Nakai.

This is a perennial plant, with annual aerial stems that are slightly woody and hollow. It is visible from late spring until the beginning of autumn, while in winter it is less obvious, as its leaves fall and the stems above ground tend to rot. It possesses large subterranean rhizomes, which can reach a depth of 3 m and have an extraordinary ability to propagate the plant by vegetative means. The aerial stems reach a height of 2.5-3 m and a diameter of 2-3 cm at the base. The leaves are roughly heart-shaped, with a sharp apex and, on average, measure from 21×18 cm to 30×23 cm. On the underside, they have a few short, whitish hairs, distributed mostly along the main veins. The inflorescences of the *Reynoutria x bohemica* are shaped like small clusters, 4-12 cm long, slightly bent towards the lower part and situated at the axil of the leaves. They carry numerous small flowers, which are white or greenish-white, in clusters of 2-7. The fruit is a dark brown achene, with a triangular outline, 2.5-3 mm long, and is smooth and shiny.

In the Tuscan stations, this species regularly blooms and bears fruit. However, the ability of the seeds to germinate is still not clear. For this reason, studies are under way at the University of Florence aimed at estimating the germination rate and thus assess the ability to propagate by seed, which would be in addition to vegetative propagation.

In Tuscany, as well as *Reynoutria x bohemica*, the two entities from which it probably originates, are also present: *R. sachalinensis* and *R. japonica* var. *japonica*. These two plants are not dissimilar, but each has unique features that make it possible to distinguish them.

The Bohemian knotweed is now common in most of Europe. The first sighting of the plant in Italy was in the lower Casentino (Arezzo) area, following which analysis on a national scale was launched, allowing for an initial delimitation of its distribution in Italy to be established[72]. The species is currently distributed in central and northern Italy (Valle d'Aosta, Piedmont, Lombardy, Veneto, Friuli-Venezia Giulia, Liguria and Tuscany[73]). It should be noted that both parent species are present in Tuscany: *Reynoutria japonica* var. *japonica* appears to be particularly invasive in the upper Valdarno area (Arezzo - Florence), where has colonized long stretches of ravines in the Valdarno plain, while *Reynoutria sachalinensis* appears to be less common and is less invasive. To date in Tuscany, *R. × bohemica* has been spotted in various provinces (Arezzo, Prato, Pistoia). From an ecological point of view *R. × bohemica* and the other parent species occupy different types of habitat: the preferred habitat are the wetlands and watercourses, but they have also invaded roadside and railway areas, parks and gardens, quarries and rubbish dumps, urban/agricultural areas, uncultivated areas and the edges of forests.

In the study area, R. bohemica has been found for the first time in Pistoia, along the banks of the Ombrone[72], in the locality of Podere della Chiesa, where it formed dense nuclei both on the outer side of the bank and in the riverside area. The inspections carried out confirmed the presence of this plant in the area mentioned, making it possible to find it along the embankment of the Pistoia side as well.
The investigations were therefore also extended to the areas upstream and downstream of the Lake Ombrone station. In the upstream area, *R. bohemica* turned out to be spread along virtually all of the Ombrone River as far as its upper area in the province of Pistoia. Its presence has been confirmed at least as far as the confluence with the Vincio di Montagnana torrent. This last watercourse has also been invaded by this species and could, therefore, represent a point of departure or at least an area of special importance due to its widespread presence throughout the area of the Plain.
In the stretch downstream of Lake Ombrone *R. bohemic*a has been found at least as far as the locality of Guado del Molin Nuovo.
In terms of size, the populations varied widely, ranging from extensive, very dense nuclei to others of just a few square metres, discontinuous and spaced out. The nuclei were seen mostly along the inner and outer embankments, in a raised position with regard to the water level, although the plant was often also found growing along the muddy banks in more or less prolonged contact with the water.

Impact
International[74] and national[72] studies have revealed a considerable reduction of biodiversity in the invasive plant communities by this and by other species of the *Reynoutria* genus. The tendency to form dense populations, with thick coverage and a dense network of subterranean rhizomes make it particularly difficult for native species inside the areas populated by the *Reynoutria* to survive. Also at the Prato station, the initial observations confirmed this tendency, with the presence of populations made up of very few species or even of the Reynoutria alone. In the invaded area, the communities most commonly in contact with the populations of Reynoutria are: dense canefields that are often mono-specific of *Arundo donax* and populations of nitrophilous and ruderal species, such as *Urtica dioica, Galium aparine, Rubus ulmifolius, Arum italicum, Persicaria lapathifolia* etc.
From the initial observations, we can state that this last type of vegetation is the one destined to be most easily invaded and replaced by the coenosis of *Reynoutria*.

BLACK LOCUST

Biology of the species and local distribution
Robinia pseudoacacia, commonly known under various names (black locust, robinia, acacia, false acacia, cascia), is a deciduous tree, belonging to the family of the Fabaceae (or Leguminosae).
It can grow to 25 m in height, although it is often found in the form of a basal shooting shrub (coppiced). The species is known for its ability to propagate through basal shoots, both roots and stumps[75]. It has a very extensive surface root system with the unusual feature (common to many Leguminosae) of being able to absorb atmospheric nitrogen, thanks to the symbiosis with specific nitrogen absorbing bacteria.
The bark of branches and young stems is smooth and greyish, while that of the adult specimens has longitudinal long, narrow, diamond-shaped splits. The young branches often have numerous large, spiky thorns. On average, the leaves are made up of 13-15 elliptical segments, 3-5 cm long, rounded at the tip, of a glaucous green colour, lighter on the underside. The flowers are in hanging clusters, 10-20 cm long. Every single flower is 15-20 mm long and is a white or tending towards a greenish-yellow colour. The fruit is a flat pod, 5-10 cm long, containing 3-10 seeds.

In its native area, *Robinia pseudoacacia* is a typical species of the mesophilous (cool and damp) woods, generally with mixed broad-leaved trees. Following its introduction to Europe, it gave rise to different ecotypes that were able to colonize both cool-damp environments with an ocean climate and also hot-dry ones with a Mediterranean climate, thereby widening its area of distribution. It prefers sunny locations and rich soils, which tend to be acidic, cool and deep; it suffers from a lack of water, while it resists well in harsh winter weather. Now naturalized and highly competitive, it forms dense, rapidly growing thickets. It tolerates pruning and pollarding very well, responding with abundant new growth.

Fig. 29 – *Robinia pseudoacacia* L.
Ordine: FABALES; Famiglia: FABACEAE

Native to North America, it was introduced to Europe in 1601 by Jean Robin, a botanist of the French royal house, while in Italy it has been cultivated since 1662 at the Botanical Garden of Padua. It was initially used as an ornamental plant and became very common in parks and along avenues; very soon, however, thanks to its ability to adapt to multiple soils and climatic conditions, its rapid growth and vigorous root system, its use became increasingly frequent and diversified. For example, it was used extensively as a pioneer plant for strengthening and controlling erosion of the soil and in reforestation works. It is also widely used for the production of firewood or wood for carpentry and as a melliferous plant. Today, it is widespread in every continent, including Africa and Australia, and it is particularly common in Europe, where it is often cultivated in large plantations.

In Italy, it is currently recognized as an invasive species in all regions except for Puglia, Sicily and Sardinia, where it is present in a spontaneous and naturalized form and where its expansion is probably limited by the dry summer weather. In Tuscany, it has been present since eighteenth century. The provinces that are currently most affected by its invasive nature are Lucca, Pistoia, Prato, Massa Carrara and Florence. It is invasive and very competitive in sparse woodlands and chestnut groves, where it forms dense woods, especially in the rainiest areas in north-west Tuscany. Elsewhere, it is common, but dispersed and confined due to the dry summer weather.

In the territory involved in the "Water SCIs" LIFE project, a limited presence of the Black locust has been found in the areas of the Park of the Cascine di Tavola (Nature Reserve of Local Interest) and at the smaller Lake Bogaia. On the other hand, the species has not been found in the remaining sites (Lake Ombrone and Lake Pantanelle).

Impact

Although it is famous for its remarkable productive potential, the Black locust is considered one of the 100 most invasive species in the world and in Europe[76] and also one of the 10 most invasive plants in Italy[77]. Although it produces abundant seeds, it renews itself mainly, and very effectively, by vegetative means. In fact, the strength of the invasive nature of the Black locust lies in its ability to regenerate rapidly and abundantly,

starting from basal shoots, and also in the nutritional advantage provided by its symbiosis with nitrogen fixing bacteria, allowing rapid growth even in soils that are poor in nitrogen. It therefore forms dense thickets that modify the structure and the range of flora in the invaded areas, as well as the chemical composition of the soil. The Black locust is one of the first to colonize open spaces and it occupies a vast number of environments, thanks to its ability to tolerate different environmental factors. Although it is mostly linked to disturbed ecosystems, it may also spread within sheltered environments and open woodlands, causing the loss of biodiversity. In coastal areas and especially on islands, it is limited by the dry summer weather, which keeps its spreading under control, but in the cooler inland areas it is able to spread with great force and speed, creating pure woods stretching for tens of hectares. As a result, the appearance of the landscape in the hilly areas of provinces like Pistoia and Lucca has changed completely in the space of a few decades.

Relationships with the stakeholders

Talks and discussion with stakeholders (environmental protection associations, hunters, fishermen, and town committees) continued for the entire duration of the project, constantly seeking effective collaboration aimed at the conservation of nature, which probably represents the most qualifying and distinguishing factor of this LIFE project.

During the first six months of 2010, three specific agreements were stipulated with the owners of the lands on which the wetlands of the Prato plain stand and which were identified in the project with the purpose of:

- Guaranteeing access to representatives from the Provincial authority for the in-depth analysis and preliminary studies;
- Authorizing the Prato Provincial Authority to perform the environmental requalification measures included in the project;
- Guaranteeing that the areas undergoing conservation measures are destined for nature preservation beyond the duration of the "Water SCIs" LIFE project.

The three wetland areas identified belong to three entities that are completely separate, even from a legal standpoint. Lake Pantanelle is, in fact, owned by a company called "G.I.D.A." S.p.A. (Gestione Impianti Depurazione Acque - Water Purification Plants Management), a joint-stock company with mixed public and private capital, which manages the water purification plants in the Municipalities of Prato, Vaiano, Vernio and Cantagallo, the sewage treatment plant in Calice and the industrial waterworks network. The wetland is run for hunting purposes (fixed hunting post n. 857 for palmipedes and waders).
Lake Ombrone, on the other hand, belongs to the Institute of religious education and moral assistance for the Youth of the Diocese of Prato, which is for all intents and purposes a private entity. Here too, the lake is used for hunting activities (fixed hunting post n. 765 for palmipedes and waders).
Lake Bogaia and the surrounding land belong to the Prato Municipal Administration. The area was involved in hunting activities in a planned form up until the approval of the Wildlife Hunting Plan of the Province of Prato (Provincial Council Resolution n. 59, dated 18/12/2013), which, by taking on board the specifications of the Management Plan of the "Ponds of the Florentine and Prato plain" SPA, established a protected area named "Bogaia", in accordance with article n. 14 of Tuscan Regional Law n. 3/94.
Lastly, the land on which the fish hatchery of Ponte S. Giorgio (Camugnano - BO) was created, was acquired by the Management Consortium of the Lakes Suviana and Brasimone Natural Park following an amicable agreement with the owner on 29 July 2010. The land-parcel acquired, which has a surface area of approximately 1,000 sqm, is identified on sheet 82, map section 121 of the New Land Register of the Municipality of Camugnano (BO).
The obligation to ensure the protection of animal and plant species targeted by the community project in the lands described, for twenty years after the conclusion of the actions performed, and to transfer this commitment to any new user, tenant or assignee in the event of transfer of the aforementioned lands, has been explicitly included in the text of the agreement stipulated with the owners mentioned and also in specific "transfer of deeds" registered at the land registry premises (Land Registry Office).
After overcoming some initial, understandable diffidence due to insufficient knowledge of the LIFE project tool and of its implications in terms of constraints, the owners of the wetlands affected by the environmental re-

qualification measures maintained a collaborative attitude throughout the entire duration of the project. This was expected in the case of the Municipality of Prato, which is incidentally one of the funding sources for the project, but it was not to be taken for granted in the case of the other two entities (GIDA S.p.A. and the Institute of religious education).

The relationships with owners of fixed hunting posts in the various locations proved to be profoundly different: they were very cooperative in one case (Lake Pantanelle), and strongly opposed in the other (Lake Ombrone).

During the implementation phase of the project, efforts were made to maintain an open communication channel with the so-called "lagaioli - lake hunters", owners of the hunting posts, particularly in the planning stages of the measures to be undertaken, bearing in mind that these are the specimens in closest and most constant contact with the territory to be requalified. Lake hunting is, in fact, a sedentary type of hunting activity that entails the long-term maintenance of environmental conditions suited to the presence of birds. For this reason, the "lagaioli" lake hunters are directly involved in managing the water levels of the bodies of water, regulating the inflow and outflow of water, as well as routine maintenance of the vegetation, in compliance with the obligations and requirements set out for the SPAs in Tuscany, by Tuscan Regional Government Resolution n. 454, dated 16/06/2008, concerning: "Minimum uniform criteria for defining conservation measures relative to Special Areas of Conservation (SAC) and Special Protection Areas (SPA)".

In the case of Lake Pantanelle, the owner of the hunting-post demonstrated a certain open-mindedness right from the earliest stages with regard to the topic of conservation of nature as a whole (and not only relative to birds from a hunting point of view), while the "lagaioli" lake hunters at Lake Ombrone, who felt that the environmental improvement measures would have a negative impact on hunting, systematically rejected every attempt at dialogue, to the point of asking that the lake be removed from the SPA.

In order to improve compliance with the correct methods of managing Lake Ombrone, to ensure the conservation of nature, specific requirements have been inserted into the provincial hunting regulations, aimed at achieving the goals of the project.

With regard to environmental campaigners, clear opposition to the project was expressed by the Tuscany regional committee of the WWF, which was firmly against maintaining hunting activities in the site of Lake Pantanelle. This position, which is legitimate and consistent with the policy pursued by the association on a national level, was, however, taken to the extreme by the local committee to the point of insinuating, in a letter sent to the "LIFE Nature and Biodiversity" Unit of the European Commission, that "community public funding devoted to the protection of biodiversity was spent by the Provincial Authority of Prato, on the one hand to obtain the possibility of more easily attracting and then killing the species of migratory birds in this site (i.e., the opposite effect of that envisaged) and, on the other hand, led to the "beneficiaries" of this funding being just a small group of hunters and, therefore, not even the entire "community" of hunters in the Province".

Although clearly unfounded, claiming that the public funding of the project was used to favour single private entities (the owners of the hunting-posts) is a very serious accusation against the public administration and reflects badly on the project, both in the eyes of the European Commission and also with regard to public opinion (given that the same view was upheld by the local media). The Provincial Authority of Prato, as beneficiary coordinating the project, has systematically rejected these allegations before the appropriate institutional and non-institutional (daily newspapers, radio) bodies. However, the best response to these unfounded accusations comes from the results of the biological monitoring carried out during the project, confirming that the current management methods used at the Pantanelle wetlands, despite being the result of a composition of non-converging interests, are clearly and actively contributing to the attainment of conservation objectives.

During the years 2012 and 2013, an informal collaboration with the associations "Via del Campo" and "Polisportiva Aurora" was established. These associations operate in the field of mental disorders and run an educational farm called "Animal house", located immediately to north of Lake Pantanelle, where domestic animals are kept and guided tours are organized. By avoiding the nesting period of the target species, the guided tours of Lake

Pantanelle have become a part of the environmental education offering that these associations present annually to schoolchildren in the territory. Similar collaboration with the association "Amici della Bogaia" was sought and obtained. This association runs Municipal "cattery" structure of the same name, near Lake the Bogaia, with the aim of maintaining the fencing that encloses it an efficient state, in order to limit potential episodes of predation of chicks and protected small wildlife by stray cats.
Lastly, the relationship with the association of fishermen "Prato Mosca Club" proved to be very positive. The first relations with this associate were sporadic and informal (telephone and email contacts), after which an intentional meeting took place, held on 04/02/2013, in the presence of the scientific advisers of the Provincial Authority of Prato (ichthyologists from the company Bioprogramm) and a representative from the Provincial Hunting and Fishing Service, to illustrate the new methods of fish propagation according to the approved species-specific action plans (sees box dedicated to you) for the Apennine watercourses in question. These methods were fully shared by the association, which manages both the stretch of the watercourse in which passages for fish have been created ("Rio Trogola no-kill area" with specific regulations), and the fish hatchery of the Union of the Val Bisenzio Municipalities in the locality of "Casa al Rio" (Cantagallo - PO), where part of the material used for propagation is produced. The association has also declared its availability, on the basis of partial payment of expenses incurred, to keep the fish passages clean, as they periodically fill up as a result of flooding or falling leaves in autumn.

Action Plans for the conservation of the European bullhead and the White-clawed crayfish
Action plans are management tools described in Article 6, paragraph 1 of Community Directive 92/43/EEC "Habitats" and Article 4, paragraph 2 of Presidential Decree n. 357/1997 that implemented the Community Directive at national level.
Regional Decree n. 1014/2009 defines action plans as: "technical documents that describe, on a very variable scale (from global level to one of very small areas), the actions required to conserve single species (e.g. the action plans produced by the ISPRA Environmental Protection and Research Institute - formerly INFS - for the conservation of threatened species of Birds and Mammals in Italy), but also groups of species and habitats. Action Plans may also be produced to define methods of managing certain human activities (e.g. wild grazing) that interfere with or allow for the protection of certain species or habitats".
In the context of the "Water SCIs" LIFE project, the plans have been geared towards reducing the threats/critical situations found and increasing the size and the vitality of the populations of species in question, by pursuing the following specific goals:

- Protecting of existing populations of *C. gobio* and *A. pallipes*;
- Recovering and reintroducing populations of European bullhead (*C. gobio*) and White-clawed crayfish (*A. pallipes*) in sites where they previously existed and currently appear to have disappeared or are very rare;
- Protecting environments and specific habitats of the species in the territorial areas covered by the plans.

The Action plans were approved by the Provincial Authority of Prato by Regional Government Resolution n. 43/2012 and by the Management entity for the Parks and Biodiversity of Eastern Emilia by Decree n. 84/2012 and have full legal effect right from the approval date. All approved Action plans can be viewed and downloaded from the project website (http://life.provincia.prato.it).

A SERIES OF INITIATIVES: "NATURAL LIFE IN THE TERRITORY OF PRATO"

The "Water SCIs" LIFE project was presented to the public for the first time on 11 October 2009 in the locality of Cascina di Spedaletto (Cantagallo - PO), as part of the initiative called "Biodiversity Day". On this occasion, a prize was awarded by the T.C.I. to the Provincial Authority of Prato for commitment shown by the Administration in the field of protecting biodiversity. A further "kick-off meeting" of the LIFE project was organized by the Lakes Suviana and Brasimone Natural Park at the ENEA (National agency for new technologies, Energy and sustainable economic development) Brasimone Energy Information Centre, in the locality of Bacino del Brasimone, Camugnano (BO) on 28 May 2010.
Citizen involvement was prolonged throughout the second trimester 2010, with a series of "Natural Life in the Territory of Prato" seminars and excursions, organized by the Department of Protected Areas in the Province of Prato and by the Natural History Museum of the University of Florence, on the occasion of and under the protection of the International Year of Biodiversity.
The initiatives saw overall participation by approximately 75 people: administrators, technicians, members of associations and committees and ordinary citizens interested in the subject. Below is the calendar of appointments and topics covered:

FIRE
- - Seminar: Thursday 15/4 at 21:00 - Geology of the territory of Prato and "Natura 2000" network (geology and Natura 2000 network).
- - Field trip: Saturday 17/4 at 15:00 - The origin of Monteferrato and its geological and floristic treasures (geology and flora).

AIR
- - Seminar: Thursday 22/4 at 21:00 - The migratory population in the plain between the Arno and the Ombrone Pistoiese (birdlife).
- - Field trip: Saturday 24/4 at 15:00 - Birdlife in the wetlands of the Prato plain (birdlife and management of the wetlands).

WATER
- - Seminar: Thursday 13/5 at 21:00 - Fins, pincers, legs: life in the rivers and torrents of the Prato Apennines (freshwater fish, amphibians, macro-invertebrates).

EARTH
- - Seminar: Thursday 20/5 at 21:00 - Little eyes in the forest and in the meadow (arthropods).
- - Field trip: Saturday 22/5 at 15:00 - The lowland forest and its inhabitants in the Cascina di Tavola Estate (athropods and invasive alien species).

The series of initiatives had a dual objective: on the one hand, to raise public awareness of the importance of the topic of biodiversity and its conservation and, on the other hand, to set up a work-group made up of representatives from associations in the territory, Organizations and individual citizens interested in the protection of biodiversity, with whom to discuss the progress of the LIFE project and identify opportunities, critical areas, strengths and weaknesses.
The work was developed by adopting S.W.O.T. analysis methodology, a support tool for the analysis of the context, consisting of the identification of strengths and weaknesses of the context itself and analysis of the opportunities and threats deriving from the external context to which the analysed project is exposed; in this first phase, with specific reference to the area of the Prato plain.
By using this approach, we were able to clearly and concisely highlight the variables that could facilitate or

hinder the achievement of the project goals, distinguishing between local and external factors.
Lastly, the S.W.O.T. analysis was integrated and completed the following year during the following events, when we also presented executive projects for the requalification measures in the wetlands in the Prato plain:
“FESTA IN PANTANELLE”, which took place on 15 May 2011 at the Sports Field of Casale - Lake Pantanelle, Prato;
“THE TREASURES OF PRATO”, which took place on 26 May 2011 at Palazzo Vestri, Piazza Duomo, Prato.

Other public initiatives

Fig. 30 – Poster of the initiative.

During the spring of 2013, three separate public initiatives were organized (one for each site where requalification took place) and saw the participation of approximately 30 people per meeting on average, with the aim of acquainting citizens with the environmental improvement measures carried out in the Prato plain as part of the “Water SCIs” LIFE project.
On Saturday 13 April 2013, the “From Animal House to Pantanelle” initiative took place, organized in collaboration with the “Via del Campo” and “Polisportiva Aurora” Associations that manage the “Animal House” educational farm located near Lake Pantanelle.
The initiative included a tour and illustration of the environmental requalification measures performed, an exhibition of photos taken at Pantanelle by two nature photographers during different seasons of the year, and “active planting” using forest propagation materials kindly made available to the project and to citizens by the State Forestry Corps.

Fig. 31 – Planting shrubs on the embankment of the lake.

On 26 May 2013, the "An afternoon with the Stork" initiative took place, organized in collaboration with the Centre for Natural Sciences of Prato, the Terna S.p.A. Company and the CSN/Gruppo Astrofili Quasar Volunteer Association. The event was given this name, as it was focused on the attraction of the return (after 300 years of absence from territory of Prato) of a nesting presence of the White Stork, not by chance located near Lake Ombrone, where the food resources available are useful for nesting and for the development of this "emblematic species".
In the month prior to the initiative, the nesting in progress could be watched via streaming on the "Water SCIs" LIFE project website, thanks to a webcam carefully installed in the vicinity, without any disturbance to the nesting pair.
A drawing contest was also held for primary school pupils in the territory: on 24 May 2013, the classes of the pupils who won prizes visited the nesting site and the environmental improvement measures carried out alongside Lake Ombrone.

Fig. 32 – Poster of the initiative.

Fig. 33 – Visit to Lake Ombrone with schoolchildren.

Fig. 34 – Poster of the initiative.

The "From the forest… to the lake" initiative, organized in collaboration with the Environmental Department of the Municipality of Prato and the Association "Amici della Bogaia" on 16 June 2013, included a guided tour of the lowland forest and the Medici Estate of the Cascine di Tavola, up to Lake Bogaia, where the LIFE project environmental requalification measures had recently been concluded.

Fig. 35 – Illustration of the measures.

Lastly, on 14 September 2013, as part of the "Expo Rurale Toscana 2013" exhibition at the Fortezza da Basso in Florence, a final conference was organized to present the results obtained by the project, called: "Co-existence between rural activities and the conservation of species of community interest: the "Water SCIs" LIFE Nature project".

The Councillor for Protected Areas in the Province of Prato, Dr. Alessio Beltrame, and the President of the Management Body of the Parks and Biodiversity in Eastern Emilia, Dr. Sandro Ceccoli, brought greetings from their relative Administrations and introduced the theme of the conference. The following presentations were then made:

The *in-situ* conservation of the fish species C. gobio and the White-clawed crayfish A. pallipes (by the Bioprogramm studio and the University of Parma);

- The *ex-situ* conservation of fish species and astacidae in the fish hatchery at Ponte S. Giorgio (by the Managing Body of the Parks and Biodiversity in Eastern Emilia and the University of Bologna);
- Actions undertaken to favour preservation and improvement of the conservation status of the populations of Triturus carnifex and of the target bird species (by the Biosfera studio);
- Actions aimed at contrasting/containing the alien species in the areas that were the object of envi-

Fig. 36 – A moment from the final convention at the 2013 Rural Tuscany Expo.

ronmental improvements (by the Nemo studio and the University of Florence);
- Protection tools: the Management Plan for the "Ponds of the Florentine and Prato plain" Special Protection Area and the action plans for the conservation of *A. pallipes* and *C. gobio* (by the Provincial Authority of Prato).

About twenty people participated in the conference, including representatives from the Institutions, from the academic world and individual citizens.

THE MANAGEMENT PLAN FOR THE SPA

Two converging reasons led to the need to draw up a Management Plan for the part of the Natura 2000 "Ponds of the Florentine and Prato plain" situated in the territory of the Province of Prato:
1. The specific requirements of in Regional Government Resolution n. 644/2004, which indicated a "very high" need for a management plan for the site in question, under the terms set out in Article 6 of the 92/43/EEC "Habitats" Directive;
2. The provision of "a precise short-medium and long term management framework for the areas of intervention and the target species (Natura 2000 Management plan for the extended "Ponds of the Florentine Plain" SPA)" contained in the "Water SCIs" LIFE project.

The Management Plan, approved in accordance with the procedure laid down by Title II of the Regional Law on the Government of the Territory (Tuscan Regional Law 1/2005), has pursued the goals of maintaining or restoring to a satisfactory conservation status, the natural habitats and the species of fauna and flora of community interest present in the site, while also ensuring the proper fruition of the natural heritage by citizens.

The Plan was approved by Municipal Council Resolution n. 50/2012 and became fully effective as of 7 November 2012, in which it was published in B.U.R.T. (Tuscan Regional Official Bulletin) n. 45.
In compliance with the provisions of Regional Council Resolution n. 1014/2009, "Tuscan Regional Law - approval of the guidelines for drafting the management plans of the SRI (Site of Regional Importance)", the approved Plan is divided into the following sections:

1) COGNITIVE section, consisting of the following documents:
   - Knowledge Base Report, which contains existing legislative, regulatory, administrative, planning, programming and contractual elements, the biotic and abiotic features of the site, with particular reference to the naturalistic ones, the factors causing persistent pressure on the site and the social and economic conditions in this context.
   - Table 1 – Map of territorial location
   - Table 2 – Hydrographic chart
   - Table 3 – Chart of flora present
   - Table 4 – Map of vegetation
   - Table 5 – Map of habitats
   - Table – 6a Map of fauna present
   - Table – 6b Map of fauna present
   - Table 7a – Chart of suitability of fauna
   - Table 7b – Chart of suitability of fauna
   - Table 7a – Chart of suitability of fauna

- Table 8 – Chart of agro-zootechnical farms
- Table 9 – Chart of soil use
- Table 10 – Chart of public properties
- Table 11 – Chart of constraints
- Table 12 – Chart of Hunting Institutes
- Table 13 – Chart of cultural heritage
- Table 14 – Map of the ecological mosaic
- Table 15 – Document of influencing factors

2) PLANNING section, consisting of the following documents:
   - Plan Report, containing management indications based on the adequate identification of ecological requirements and of problems inherent to the species and habitats present. These indications were aggregated, according to their content, into the following categories:
     - Active interventions;
     - Regulations;
     - Incentives;
     - Monitoring and research;
     - Educational programmes;
   - Table 16 – List of actions.

3) INTEGRATED ASSESSMENT Section, consisting of the following documents:
   - Summary Report of the Integrated Assessment, with contents provided for under Article of Regional Government Presidential Decree n. 4/r/2007 and the relative attachments;
   - Attachment 1 – Assessment of Internal Coherence;
   - Attachment 2 – Assessment of External Coherence;
   - Attachment 3 – Assessment of the environmental, social and economic effects and effects on human health.

The approval procedure used allowed the full participation, in the formation of the Plan, of all public and private entities interested, as set out in more detail in the next paragraph. All documentation of the approved Plan can be viewed and downloaded from the project website (http://life.provincia.prato.it).

Public participation in the management plan for the SPA

Public participation in the drafting of a Management Plan, as with all tools used for planning or government acts in the territory, is undoubtedly a good administrative practice, useful for collecting contributions and indications from all stakeholders and for identifying and avoiding any controversies or conflicts.
Regional legislation thus provides specific operative and procedural methods: the approval procedure for the Management Plan, as clarified by Regional Council Resolution n. 1014/2009, “Tuscan Regional Law 56/00 - approval of guidelines for the preparation of management plans for the SRIs (Sites of Regional Importance)”, does, in fact, follow the procedural norms dictated by articles 15, 16 and 17 of the Regional Law for Government of the Territory (Tuscan Regional Law 1/2005), for the approval of territorial planning tools (the so-called “unified procedure”).
This unified procedure consists of the following phases:

1) Initiation of the procedure - (act approved by the Provincial Administration, through Provincial Government Resolution. n. 191/2011): the document launching the procedure contains, among other things, a definition of the goals of the plan, consequent actions, the environmental and territorial effects expected and the knowledge base of reference. It was sent to the entities indicated in Regional Law 1/2005, including the Municipalities and the Provincial Authorities involved, the Tuscan Regional Authority, the Mountain Community, the Superintendence, ARPAT (Regional Environmental Protection Agency of Tuscany), the Land Reclamation Authority of the Ombrone and of the Florentine Area, the Arno Basin Authority, the ASL (Local Health Authority), ISPRA (Environmental Protection and Research Institute), the Regional Office for the Protection of Water and of the Territory and the CFS (SFC - State Forestry Corps).
The document was sent to these entities with the aim of integrating the initial knowledge base of reference with technical contributions and to gather every observation, opinion, information and contribution that could assist in drafting the Plan.
Adoption - (act approved by the Provincial Administration in Provincial Council Resolution n. 18/2012): the adoption deliberation, with the relative documents (specifically, a definitive proposal of the Plan to be approved) was transmitted to all institutions involved and the notice of adoption was published in Tuscan Regional Official Bulletin (B.U.R.T.) n. 20 on 16 May 2012.
The Plan adopted was also filed with the Provincial Authority of Prato for sixty days from the date of publication of the relative notice in the B.U.R.T. (Tuscan Regional Official Bulletin) and the documents were made available to all citizens on the "Water SCIs" LIFE project web page, allowing anyone to view it and submit comments. The period of sixty days from the date of publication in the B.U.R.T. expired on 17/07/2012 and the comments received were collected and examined, in order to respond as necessary.
Approval (Plan approved by the Provincial Administration, in Provincial Council Resolution n. 50/2012): the approval measure, containing precise reference to the comments received and the explicit motivation for the decisions subsequently adopted, was communicated to the institutional entities involved and was made accessible to all, also in electronic format.
The notice confirming approval of the territorial planning tool was finally published in the B.U.R.T. (n. 45, dated 07 November 2012) and came into effect on the publication date.

In view of the adoption of the Plan, a meeting was convened, with active participation open to citizens, in order to verify the completeness and correctness of the project contents and to gather contributions and suggestions from the stakeholders invited (institutional entities, representatives of the civil society, private citizens). The meeting was held on 09 February 2012, in the presence of representatives from the Provinces involved (Prato and Florence), of Administrators and technicians from several Municipalities involved, as well as representatives from local associations involved and private citizens, including trade associations from the agricultural sector. It also helped to increase awareness on this topic and operational participation of entities directly involved in the Plan, like members of the Golf Club (located in the area of the Cascine di Tavola) and the owners of the fixed hunting posts in the territory involved by the Plan.
All the documentation relative to the Plan (administrative acts and documents) has been published on the project Internet website: http://life.provincia.prato.it.

Environmental requalification of the wetlands

The planning activities for environmental requalification measures for the wetlands were all carried out in the same period: from mid-November 2010 to mid-May 2011. As well as the appointed contractors, project experts in charge, consultant experts in nature conservation were also engaged, who clarified the ecological needs of the species of interest, thereby ensuring that the projects would respond in full to these requirements.
Similarly, the planning of the fish hatchery at Ponte San Giorgio (Camugnano - BO), carried out in the third and fourth quarters of 2010, was supervised by ichthyologists appointed by the Lakes Suviana and Brasimone Natural Park. Lastly, the planning of the fish passages was carried out from September 2010 to March 2011 by project designers selected on the basis of their specific ichthyological expertise and their knowledge of the river environments involved in the interventions.

Lake Pantanelle
The planning of the intervention at Lake Pantanelle adhered to the following specific objectives:

- Implementing activities of intake and restitution relative to regulation of the incoming and outgoing flow of water to and from the lake;
- Creating a micro habitat with characteristics suited to the needs of the target amphibians and birds;
- Creating activities to mitigate the current and the potential impacts caused by roads being built alongside the western shore of the lake.

Work was handed over to the contractors on 14 July 2011 and completed on 9 January 2012.
The regulation of the water levels had previously been carried out using precarious systems, completely unsuitable for guaranteeing adequate management: to allow for a more flexible and reliable regulation of the depth of the lake reservoir, an intake activity (surface water diversion) at Fosso Calicino and a drainage activity at the lake downstream were constructed.
The new intake activity was achieved by creating a concrete threshold on which removable metallic profiles were installed, functioning at the moment of operation of the barrier structure made of sheets of wood and controlling water flow. This structure is also manually removable and is intended to remain in the bed only for the time necessary to carry out the diversion. The implementation of the new intake activity was completed using protection reefs upstream and downstream of the threshold, to avoid processes of erosion, considering that the flow rate at Fosso Calicino is abundant for a good part of the year (see Fig. 37). The characteristics of the intake activity were created to guarantee the maintenance of an adequate minimum vital flow into the Fosso Calicino, downstream of the activity.

To further regulate capacities coming into the lake, a floodgate was also created (see Fig. 38), installed at the mouth of the diversion duct in PVC, passing underneath the ring road currently under construction.
As for the reinstatement of the previously existing drainage duct on the side south of the lake, a duct was installed under the southern embankment of the lake (see Fig. 39), that delivers the drainage waters into the surrounding network of ditches, finding its final outlet in the Calicino. The drainage duct is also equipped with a special regulating floodgate.

For the environmental improvement of the lake, primary importance was focused on the partial mitigation of the negative impacts of the new road being built on the west side (second ring road in Prato). To this end, a shield

Fig. 37 – View of the intake activity and relative protection reef.

Fig. 38 – View of the entrance floodgate.

was created by planting a buffer zone of native plant species including trees and shrubs.
The new ring road project includes maintaining a service road with an average width of about 5 m, located at the base of the road embankment, along the west bank of the lake. At the edge of this service road, at a distance of 4 m from the foot of the road embankment, a large wooden barrier was installed to separate the area of the lake from the gravel service road and channel the smaller fauna towards the underpasses already built along the ring road, in order to limit the interference of vehicle traffic with the movement of fauna, both during the building phase and when the road is operational, thus reducing the risk of collisions. Vegetation was planted along the entire area between the wooden barrier and the west shore of Lake Pantanelle (see Fig. 40).

Fig. 39 – View of the return floodgate.

Fig. 40 – Creation of the plant buffer zone.

The species planted were tree and shrub forms. In particular, hygrophilous species of the *Salix* genus were used, as well as species from the more rustic *Ulmus* genus, alongside suitable hygrophilous shrubs such as the common dogweed (*Cornus sanguinea*) and the guelder rose (*Viburnum opulus*, planted alongside more thermophilous plants: common hawthorn (*Crataegus monogyna*) and cornelian cherry (*Cornus mas*). The vegetated strip was completed and integrated by the installation, along the side facing the body of water, of a strip reed bed. The hygrophilous trees were planted near the lake shores, while the mesophilic trees with greater rusticity were planted farther away from the wet area.

Another area of the lake that needed shielding by plants was the south-eastern part, located near a recently

Fig. 41 – Central island and outcrops.

renovated building used as a private residence. Trees and a copse of bamboo were already present in this area. alien vegetation was removed and replaced by shrubs and trees similar to those used on the west side of the lake (along the ring road), in order to create a solid barrier of plants capable of further reducing disturbance from the outside.

In the central portion of the lake, we also created an island planted with native hygrophilous trees (willows and poplars) to accommodate a colony of ardeidae (heronry), in order to encourage this group of species, which are worthy of conservation in the "Ponds of the Florentine and Prato plain" SPA and whose presence could facilitate the biological control of the Louisiana crayfish population that was detected at Pantanelle during preliminary studies.

In addition to the main islet, within the surface area of the lake, on the east side, 3 smaller islets were created. These are elongated in shape, with a height above the average water surface of 0.2 - 0.3 m, and are designed to create a suitable environment for conservation priority bird species to stopover, feed and nest. The central island and the outcrops are shown in the photo below.

The area of wet meadows and reed beds currently present on the north side of the lake has been further extended to the west. Its bed has also been remodelled, creating a series of areas with water heights of a few centimetres, interspersed with areas that are generally dry.

In the area to the north-northeast, 2 amphibian nurseries were created, functioning as a source area for incrementing the number and size of the amphibian species present. These ponds have a maximum depth of 1.8 m, with gently sloping shores (average slope varies from 1:3 - 1:4) towards the deeper areas, so as to create the right conditions for amphibian motility. Following the advice of herpetologists who carried out similar measures in the ponds of the Florentine plain (Nature Reserve of Local Interest of Querciola - Sesto Fiorentino), the areas are supplied solely with rainwater (to avoid the arrival of carnivorous fish, whose food resources include the eggs of amphibians) and are physically separated from the main lake by a small embankment of hard-packed earth, on top of which there is a wooden barrier approximately 0.4 m in height, acting as an obstacle to stop predators from the lake (particularly *Procambarus clarkii*) from entering the nurseries. In fact, unlike amphibians, the astacidae species with their rigid exoskeleton are unable to climb over even low barriers, as long as they are higher than the animal's own length. On the side of the barrier facing towards the ponds, an earth mound was built to act as a sort of "slide" or "ramp".

Fig. 42 – Nurseries for amphibians.

The resulting separation structure facilitates the movement of fauna from the ponds towards the lake and limits the inflow of water from the lake (including carnivorous fish species, given that water comes from the hydraulic network with a smaller surface area). In one of the two nurseries, protective wire mesh was also set up over the body of water, to avoid the predation of the amphibians by the bird fauna present, particularly during the more delicate phases of early development (see Fig. 42).
The invasive alien plant species species present were, fortunately, very few in number, probably as a consequence of the transportation of inert materials and earth for the road works under way, and were removed from the area.
The contrasting actions against the invasive alien plant species continued on into 2013, with the experimentation of various control methods, which will be discussed in greater detail in the relative paragraph.
The surface area suitable for the recolonization of target species, following the intervention, is quantifiable as 6.2 ha (extension of the body of water).

Lake Bogaia

The environmental requalification project at Lake Bogaia adhered to the following specific objectives:

- enlarge the body of water of Lake Bogaia inside the Cascine di Tavola (Prato) protected area of local interest;
- create a micro habitat with characteristics suited to the needs of the target amphibians and birds.

Work was handed over to the contractor on 27 September 2012 and ended on 22 July 2013.
Firstly, reclamation of the area was carried out, to remove the large amount of refuse that had accumulated there, as the area is easily accessible to motor vehicles (see photo below).
The area of the lake was expanded until a project surface area of approximately 1 ha had been attained, creating new areas with different water heights and variable depths in order to encourage the simultaneous presence of various taxonomic groups, including diving ducks (e.g. the Little Grebe).
The lake was enlarged in order to obtain a main island in the centre of the new lake (see photos below), on which hygrophilous Salix and Populus tree species were planted, along with a secondary islet, which is smaller and without vegetation. These islands and outcrops are useful shelters and suitable places for the nesting of bird fauna, as they are protected from predation from the ground (see photo below).



Figg. 43-44-45 – Refuse present in the area of intervention.

Regarding the regulation of the internal surface waters in the area of intervention, the minor network already present on-site was left substantially unchanged, which was made up of a main trench and small furrows flowing into the existing lake.
Three ponds were also created around the perimeter of the main body of water, with shores gently sloping towards the deeper areas (max. 1 m), in order to create an environment that favours the motility of the amphibians. Protective netting was placed over two of the ponds, (with an opening part to allow for inspection and monitoring) with the goal of limiting predation of the amphibians by bird fauna.
The pond beds were waterproofed using geo-synthetic materials, covered by a layer of three-dimensional geomat, which was also covered with topsoil, to avoid provoking negative visual impacts and to facilitate the movement of the herpetofauna to and from the ponds.




Figg. 46-47-48-49 – Remodelling of the lake bed and the creation of islets for nesting.



Figg. 50-51-52 – Creation of pond-nurseries for amphibians.

Once the movement of earth required for enlarging and reconfiguring the lake and the ponds for the amphibians was finished, plant species were planted: hygrophilous plants were planted near the drainage net and the ponds, while those with greater rusticity were planted in the areas further away from the water. On the littoral strip of the newly created body of water, along the south-east bank, helophyte vegetation (mainly *Phragmites australis*) was planted over a strip with an average width varying between 4 and 6 m. This strip helped to isolate the internal habitat and limit the interference of possible negative factors from outside, as well as creating site conditions suitable for the shelter and nesting of bird fauna.

Along the west side of the lake, around the amphibian ponds, a small hygrophilous thicket was created, where species of the Salix, Populus and Fraxinus genus, typical of lowland forests, were planted, as well as species with greater rusticity of the Ulmus and Acer genus. Alongside these, shrubs were planted, including the common dogwood (*Cornus sanguinea*), the guelder rose (*Viburnum opulus*), the common hawthorn (*Crataegus monogyna*) and the common hazel (*Corylus avellana*). This green solution helps to increase ecological diversity in the site of intervention and its characteristics are particularly attractive to ardeidae.

Along the external perimeter of the area of operation, on the south-west side along the Via di Bogaia road and on the south-east side on the edge of the area of operation, a vegetated strip was planted with trees and shrubs, delimited by an embankment made of hard-packed earth, to create a natural defence against the disturbance caused by traffic of vehicles along the Via Bogaia and shielding the area of operation, thus increasing ecological diversity in the area. Species with greater rusticity of the Ulmus and Acer genus were used for this purpose and, alongside them, shrubs like the common hawthorn (*Crataegus monogyna*) and the common hazel (*Corylus avellana*) were planted.

During the work in progress and inspections of the worksite, stray cats were noticed in the area, due to the presence of the “cattery” or “feline oasis” located near the south-west border of the area of intervention.


Figg. 53, 54 – Planting trees and creating a reed bed.

Figg. 55, 56 – Creating a barrier and planting in rows.

Following talks with the managers of the cattery, which is the property of the Municipality of Prato, an area was identified outside the existing fence, where a small colony of semi-stray cats was living habitually (see Fig. 57). It was therefore decided fence off the area using special solutions (tilted poles and a sheet of “anti-grip” fibre-glass - see photos below), allowing the cats to enter from outside while stopping them from leaving.

Fig. 57 – Feline colony outside the cattery.

Figg. 58, 59 – The new fence.

Lake Ombrone

When planning the activities at Lake Ombrone, our primary goal was to protect and requalify the environment in the northern part of the wetland area, characterized by the presence of wet meadows, thereby creating a precious habitat, especially for the species of so-called "shore birds", united by their feeding habit of searching for invertebrates and other small animals in the shallow waters or in the mud.

The environmental improvement work at Lake Ombrone was handed over to the contractors on 5 March 2012 and completed on 4 May 2012.

One of the main factors disturbing the stability of the target species of the lake is the ease of access to the lake shores from the embankment of the nearby Fosso Ficarello, which is often used as a pedestrian pathway, with or without dogs in tow. For this reason, planting has been carried out on the north-west banks in order to obtain a green belt, approximately 3-4 m wide, of helophyte vegetation with a shielding effect. This "green curtain" contributes to the visual and acoustic isolation of the internal habitat (lake) and, at the same time, it offers a possibility of shelter to the bird species present there, thus making the habitat more attractive. The photo below shows the planting phase of the *Phragmites australis*.

Fig. 60 – Creating the reed bed.

Figg. 61, 62 – Nurseries for amphibians.

In order to create conditions encouraging amphibians to reproduce and stay in the area, two small ponds (nurseries) were built, with maximum depths of 1.5 m, surrounded by newly planted shrub vegetation. The ponds have gently sloping banks (the average slope varies from 1:3 - 1:4) towards the deeper areas, to create an environment that favours the motility of the amphibians. Taking inspiration from what was already done at Pantanelle, the preference was to concentrate the two ponds into the same area, so as to be able to delimit them both with a single wooden barrier, approximately 0.4 m in height, to prevent access to the nurseries of potential predators from the lake itself (particularly the *Procambarus clarkii*). The barrier was put into place at the top of a small embankment on the ground, to avoid any water from flooding in from Fosso Ficarello, which would also bring with it the eggs of carnivorous fish, predators of amphibians. The nurseries are supplied with rainwater and their beds were waterproofed using geo-synthetic materials: HDPE membrane covered with a three-dimensional geomat, to facilitate maintenance of the earth above the geo-synthetic material (see photo below).
On the north-west side of the lake a small hygrophilous thicket was created (see Fig. 63) and was populated both with hygrophilous species of the *Salix, Populus* and *Fraxinus* genus, typical of the lowland forests, as well as species with greater rusticity of the *Ulmus* and *Acer* genus. This area helps to increase the ecological diversity of the project site, providing shade to the "nurseries" and creating an attractive environment for the ardeidae. Inside the thicket, a network of small trenches was created, obtained by reshaping the drainage network already existing in the area and able to provide a sufficiently persistent, functional water supply to the hygrophilous forest plantation. The hygrophilous plant species were, in fact, planted near the drainage network of the ponds, while the more rustic plant species were planted in areas further away from the waters.
The trees were combined with shrubs, particularly hygrophilous shrubs like the common dogwood (*Cornus sanguinea*) and the guelder rose (*Viburnum opulus*), placed alongside more thermophilous shrubs (common hawthorn, *Crataegus monogyna*).
The soil from the digging of ponds and reshaping of the small trenches was re-used on-site, both for the embankment created around the pond-nurseries and also for remodelling the slope of the banks on the north-east side of the lake, where the reed bed buffer strip was created.
In the area that was the object of intervention, there were no invasive alien plant species found. However, in the immediate vicinity (embankment of the Ombrone river) there was an abundance of a poligonaceae (*Reynoutria x bohemica*), which represents a risk factor for the integrity of the site and should be kept under careful observation. The species has been the object of experimentation of various control methods, carried out during 2013, better described in the relative paragraph of this volume, dedicated to the counter measures against invasive alien species.

Fig. 63 – Creating a small hygrophilous wood.

Ex-situ REPRODUCTION OF THE EUROPEAN BULLHEAD AND THE WHITE-CLAWED CRAYFISH

Experimentation of methods for the *ex-situ* reproduction of the *C. gobio* and *A. pallipes* target species was carried out to estimate the effectiveness of such methods of artificial reproduction, with the goal of consolidating existing populations of the two species and to improve of the conservation status in the portion of Apennine territory involved in the project. Before capturing breeding stock from the 2 species in the natural environment for reproductive purposes, we performed:

- a detailed study of the historical data available on fish fauna in the Province of Prato;
- preliminary monitoring conducted during 2010;
- genetic typing.

All of these activities allowed for careful choices to be made when identifying the populations considered most suitable for *ex-situ* ichthyogenic practices.

With regard to *C. gobio*, the results obtained from the analysis of mitochondrial sequences carried out at the University of Parma did not reveal any differences between the populations present on the Adriatic and the Tyrrhenian sides of the Prato Apennines. This observation was confirmed by the analysis carried out at the University of Bologna, based on which, the differences found appear to be of sub-specific level and the populations examined can be traced back to the *C. gobio*[78,79] species.

Therefore, the choice of the most suitable breeding stock for *ex-situ* reproduction and of the areas in which the fry will be freed can and must be based exclusively on demographic and not genetic criteria.

Genetic analysis conducted at the University of Parma also confirmed that the specimens of *A. pallipes* caught belong to the native Italian species of White-clawed crayfish. Here too, no differences were found that would lead

us to assume that a different phylogenetic origin exists between the populations of Crayfish of the two Apennine torrents on the Prato side, from which the specimens were extracted and analysed. For the populations on the Bologna side, because of the small number of specimens found, statistically insufficient to support the principles at the basis of molecular investigations, it was not possible to carry out an in-depth analysis of the phylogenetic relationships between the existing populations in the area being studied.
As already suggested for the European bullhead, with regard to the *ex-situ* reproduction of the White-clawed crayfish, we decided that the choice of suitable breeding stock and places in which to carry out the repopulation should be based on demographic rather than genetic criteria, with the sole objective being that of protecting the already diminished populations in the territory and avoiding the creation of imbalances of any type.
At the start of the experimentation, the *ex-situ* reproduction of the target species involved the use of the system set up at the experimental breeding centre for fish species at risk, created by the Department of Evolutionary and Functional Biology of the University of Parma. The two species were housed separately while awaiting the creation of the fish hatchery at the Limentra torrent, which became operational in September 2012.

Building the fish hatchery

The works for the creation of a fish hatchery for the target species of *C. gobio*, and *A. pallipes* in the locality of Ponte S. Giorgio (Camugnano - BO) were handed over to the contractors in mid-December 2010. The structure was completed during spring-summer 2011 and inaugurated on 22 September 2011 (see photo below).
The project entailed the following activities:

- Designing a system for drawing water from the Limentra torrent to supply the hatchery;
- Building a prefabricated structure for storing the tanks, in which to perform research and breeding activities;
- Creating a breeding line with 6 tanks made of fibreglass and feeding and drainage devices.

The water supply to the fish breeding farm is guaranteed by a well of medium diameter, achieved through perforation and with the aid of muds and a covering of pipes in vibration-compressed concrete. On ground level, a reinforced load-bearing concrete slab was created for closing the top of the well and a steel cover was fastened to it, with two doors, allowing access to the well compartment and extraction of the pumps.
The depth of the well is 8-10 m and it is located at the back of the prefabricated structure that accommodates the breeding farm, between this and the Limentra torrent. From the hydrogeological analysis conducted and following the surveys carried out, the extractable rate of flow from the well proved to be approximately 4-5 l/s, which is, therefore, sufficient to satisfy the water requirements necessary for the normal operation of the fish breeding farm.
The structure that accommodates the breeding lines provides shelter for the tanks, as well as allowing research and environmental education activities for schoolchildren.
We chose to create the breeding farm inside a wooden prefabricated structure on a reinforced concrete foundation slab. The structure was built with load-bearing walls and spruce plywood and has windows and doors, all organized inside a single room where the tanks are kept and in which visitors are received; it also includes a toilet with access for the disabled, as well as a technical room where the electrical panels and service materials are stored. The building also has a filter zone at the entrance, consisting of an external porch at the main front entrance.
With regard to the tanks for breeding the two target species of the project, good use was made of the technical and scientific information acquired during the specialized conventions held at Legnaro (PD) on 15/10/2010 and at Sansepolcro (AR) on 12/11/2010. Here, in-depth discussions with experts of the field took place, on the subject of the most suitable techniques, tools and materials for the type of breeding farm to be built. In this way, know-how was acquired with regard to the breeding of the *C. gobio*, starting from the protocol developed for this purpose by the Department of Evolutionary and Functional Biology of the University of Parma, which envisages the use of special systems of tanks that function with a closed circuit and are equipped with a recirculation pump, a UV system, an automatic system for regulating ozone, a skimmer, a refrigerator, a biofilter and a system

Fig. 64 – Inauguration of the fish-hatchery in Ponte San Giorgio (Camugnano - BO).

for regulating the photoperiod. The networking with other experts of the LIFE "CRAINAT" (LIFE08NAT/IT/352) project turned out to be very useful. They provided very useful advice both in the planning phase and also in the phase of management of the structure, particularly for the breeding of the White-clawed crayfish.

The module for the controlled reproduction of *C. gobio* in the initial phases of experimental production at the experimental centre for the reproduction of fish species at risk of the University of Parma consisted of:

- Tank;
- Biological Filter with coral and plastic bio-media;
- Pump for recirculation of the water;
- UV sterilization system;
- Ozone sterilization system;
- System of continuous control of redox potential and ozone regulation;
- Eco-mix skimming system;
- Refrigeration system of 0.75 kW with automatic control;
- Photoperiod regulating system;
- Lighting system.

Fig. 65 – Well supplying water to the hatchery.

Fig. 66 – The structure that accommodates the hatchery.

The breeding farm, created on the basis of previously experimented breeding experiences, made it possible to control the main environmental parameters (light and temperature), as well as guaranteeing adequate water quality. The volume of the tank, including the biofilter, is approximately 1,100 litres and can be operated via closed circuit, with a daily turnover of 0.1% of the total litres in the system. Most of the volume lost daily can be attributed to the protein-skimmer that eliminates dissolved proteins, as well as a small volume of water (approximately 1 litre/day).
Once every 7 days (i.e. once a week) it was necessary to change approximately 30% of the water present, that is, about 350 litres, in order to avoid the excessive accumulation of nitrates: in fact, the system was not equipped with a denitrification system. When changing the water, well water was used.

The biological filter, created with coral gravel and plastic material, functioned correctly and there were no episodes of mortality linked to an excessive level of ammonia. In fact, it is important to remember that the calcareous coral substrate causes high levels of water hardness, making the presence of ammonia particularly dangerous, according to the formula: $NH3 + H2O = NH4^{+} + OH^{-}$. The decision to use a coral substrate was also dictated by the geological characteristics of the valleys of origin of the breeding stock.
The tank was set-up with a bed of torrent gravel and shelters suitable for spawning. The temperature of the water was set at 10°C ± 1°C and the redox potential at + 260 mV. In this first phase, the natural photoperiod was used.
During the production cycle, the same parameters used for the breeding of trout were used, indicated in the table below. In fact, the Sea trout and the European bullhead inhabit same environments and, therefore, it was assumed that they share the same requirements with regard to the quality of the waters.

Parameter	Concentration mg/l
Ammonia	< 0,0125
Carbon dioxide	< 20
Total hardness	> 100
Nitrites	< 1
Nitrates	< 0,1
PH	07/08/14
Dissolved Oxygen	> 60 %

Tab. 4 – Chemical characteristics of the waters suitable for breeding trout (threshold values80).

The bottom of the tank was covered with about 3 cm of river gravel from a nearby olive mill, after careful washing, and shelters were added, made from pieces of roof tile, cut into lengths of approximately 15 cm.
18 shelters were place inside the tank and two pumps were added, of the type used in the filters of aquariums, to increase the speed of the current inside each tank.
The first experimental production cycle of *A. pallipes* was also carried out in the same centre at the University of Parma, using a separate breeding line from that previously described.
The system was structured to allow easy management of the specimens, protecting the territorial dominance hierarchies, and functioned with closed circuit recirculation tanks in controlled conditions at a temperature of 8/10°C and photoperiod.

Figg 67, 68 – Breeding farm set up at the hatchery of Ponte S. George (Camugnano - BO).

Experimentation of a protocol for the breeding of the European bullhead

On 21 April 2010, the breeding stock specimens of the target species were captured and moved into the tank, which was set up and had already been operational for several days.
As far as *C. gobio* is concerned, the number of breeding stock specimens extracted was such that it would not determine any negative effects on the populations of the single torrents. Adult specimens were chosen and, in particular, mature females with a swollen abdomen, due to the presence of eggs, which were therefore close to spawning, while the males displayed a black colouring, typical of the reproductive period.
The number of *C. gobio* extracted and taken to the hatchery for *ex-situ* reproduction consisted of 51 specimens, composed of 20 females and 31 males, all subjected to genetic analysis.
The specimens of both sexes were identified based on their: scaly outer layer, head size and presence of a swollen abdomen. Once captured, the fish were transported and housed inside the production line set up at the experimental centre of the University of Parma.
The breeding stock was fed with the larvae of the common flesh fly, earthworms and frozen chironomids. No episodes of mortality occurred, neither immediately after capture, nor when housed. During transportation, the necessary instruments and measures were used to minimize possible factors of stress for the animals captured.
Although genetic investigations did not detect any differences between the Tyrrhenian and Adriatic populations of European bullhead, it was decided that the 2 genetic lines should be kept separate and, in consideration of the insufficient space available at the farm, to breed only the specimens of C. gobio coming from the Tyrrhenian sector.
After nearly a week from the arrival of the breeding stock, the first spawning activity took place, demonstrating that the previously set up environment was suitable for the ecological requirements of the species. In total, spawning took place 6 times, with an estimated total of approximately 1,000 eggs, spread over one week.
In the same period, an aquarium was also set up for the hatching of eggs, and was connected to the tank containing the breeding stock. An aquarium pump was also connected, with an overall capacity of 1,000 l/hour, while the outflowing water was channelled directly into the biofilter.
The eggs, measuring 2-3 mm in diameter, appeared as a compact mass, straw yellow in colour and tenaciously attached to the substrate. The male defends the nest, as already noted in the bibliography.
The procedure adopted was, therefore, to move the single substrates containing the eggs, approximately 1 week after spawning. The eggs were moved after one week, in order to avoid damaging the embryo during

initial development phases. The substrates containing the eggs were placed on “feeding mats” in order to allow the larvae, once hatched, to enjoy a sheltered environment, similar to what is likely to happen in nature. In some cases, during manipulation, part of the eggs became detached from the substrate and was placed directly onto the artificial substrates.
During the phase of embryonic development, however, some of the eggs became infected by a common mycosis, called saprolegnia. Unfortunately, when this fungus infects the spawn, it is very difficult if not impossible to eliminate it, because its hyphae continue to grow inside the cluster where it is impossible to intervene. Therefore, in order to limit its spread, it was decided that mechanical cleaning should be carried out and that the infected eggs should be removed, as is commonly done with salmonids. Moreover, the temperature of the water was increased by 1.5°C in order to accelerate the development and hatching of the eggs.
The saprolegnia killed off part of the eggs and, for this reason, only some of them hatched regularly on 11 May 2010: a total of about 500 European bullheads were born.
During the incubation phase, the temperature of the water was kept at 11°C and hatching took place after 15/18 days. This confirms the observations previously carried out, indicating approximately 160 degree days for the eggs to hatch.
Once the yolk sac has been reabsorbed, feeding began with the nauplii of *Artemia salina* (Brine shrimp), obtained from eggs that hatched according to normal breeding methods (salinity 30-35 ppt, water temperature 28°C, 3 gr of cysts/litre).
The fish fry immediately started to actively feed on the nauplii that had been freed into the weaning aquarium. The *Artemia* was fed in rations of 3 - 4 times spread over the day, according to the availability of staff inside of the farm. The cysts were administered each time, until the fry were completely full.
About 60 days after hatching, at a length of 15/20 mm, mixed feeding was started using Brine shrimp nauplii and finely ground, frozen chironomids. The chironomids proved to be very appetizing to the European bullheads. 65 days after hatching, feeding with Brine shrimp was interrupted in favour of a diet made up of chironomids and frozen mosquito larvae.
With the aim of making breeding conditions simpler and more standardized during experimentation, important modifications to the previously developed and used breeding protocol were made. The most important was that of removing all the gravel used as a substrate in the two tanks with adult subjects. This made it possible to carry out better cleaning, without accumulating food and excrement on the bottom. The specimens of *C. gobio* did not show any difficulty in adapting to this new breeding condition.
Moreover, perforated bricks were placed in the tank because they make it possible to accommodate a larger number of specimens within the same surface area, allowing us to increase the breeding density.
The adult specimens were fed daily with a mixed diet consisting of frozen chironomids, earthworms and common flesh fly larvae.
In the same way, no substrate was provided to the young European bullheads, to facilitate cleaning of the tank.
With regard to the newly born specimens, substrates made with simple plastic tubes, instead of perforated bricks, were tested. The tubes, in fact, allow better and simpler cleaning of the tank and they themselves are also easier to clean and disinfect. Once placed in the tank, the new substrates were immediately colonized by the fish. During this period of activity, it was possible to see how the adult specimens have extremely social and non-territorial inclinations and this facilitates their breeding.
On 28 July 2010, the specimens of *C. gobio* produced *ex-situ* were introduced into several public bodies of water in the Province of Prato, duly authorized by the Hunting and Fishing Service of the Administration concerned.
In particular, the water bodies in which the introduction of the fish took place were:

- Rio Ceppeta in the locality of Cantagallo (103 specimens);
- Trogola-Alto Bisenzio in the locality of Mulino della Sega (237 specimens).

The difference between the number born (approximately 500) and the number of specimens introduced into the environment (340) was due to the physiological mortality that occurs during the growth phase following hatching, which is significantly lower than that occurring in nature, and is aligned with the mortality rates found in artificial reproductive cycles, for this and for other species involved in farm breeding. The introduction took place in each of the two torrents, in areas with limited current speed and protected from the main flow, in order to minimize problems relating to adaptation to the natural environment as far as possible. In each torrent, we chose to carry out the introduction by distributing small groups of European bullhead fry in separate areas, diversifying the distribution, in order to increase the probability of success.
This first cycle of experimentation of captive breeding of the European bullhead made it possible to develop specific and articulated know-how, consisting of well-defined guidelines, sure to be successful, used and further perfected in subsequent reproductive cycles.

The same protocol was later implemented in the structures of the fish hatchery at Ponte San Giorgio (Camugnano - BO), in two different reproductive seasons (2012 and 2013).
The park of breeding stock used for *ex-situ* breeding, identified on a demographic and bimolecular basis (investigations conducted as part of the LIFE Project in collaboration with the University of Bologna - Department of Experimental Evolutionary Biology), proved to be easy to find in the Limentra di Treppio stretch of torrent next to the hatchery, which is home to a population of European bullhead that is well-structured in the different age groups and is abundant and in a good state of conservation.
The breeding stock adapted well to the breeding tanks, where they were fed on a diet based on earthworms, common clothes moths and frozen Chironomus larvae. The day after capture, the European bullheads were already exhibiting normal elusive behaviour, sheltering beneath the tiles, inside the perforated bricks and under the PVC outlet tubes of the tanks, showing sociable, non-territorial behaviour. At the end of the various breeding stock cycles, no particular pathologies were recorded. The specimens captured maintained a good state of health. All losses proved to be entirely physiological and the number was very limited, based on the breeding conditions.
During the first breeding cycle experienced in the newly created hatchery (2012) several difficulties occurred, linked to the malfunction of the farming system in which the breeding stock specimens were housed. On the one hand, these difficulties were caused by a loss of water from the tank's biological filter with subsequent emptying and interruption of recirculation and, on the other hand, by the breakdown of the cooling system. To avoid jeopardizing the on-going reproduction attempt, testing of the plant design and installation connected to the breeding tanks were promptly scheduled and carried out in July 2012, after the breeding activities had ended.
In order to avoid continuous deactivation of the recirculation system and to try to prevent sudden rises in temperature of the water in the breeding tanks, two outlet taps from the water supply well were opened so that the water could slowly run into the tanks, making them work continuously. Following the launch of this mode of operation, the European bullhead breeding stock in the two tanks showed no obvious signs of malaise. During the first year, in tank n. 1, the eggs of several females were spotted spread out under the two tiles; in tank n. 2, on the other hand, the females preferred to lay their eggs inside the holes of the perforated brick, where 5 heaps of eggs were found, while there was no sign that any spawning had taken place under the tiles inside the same tank. The smaller holes probably provided more shelter and greater control of the nest.
Unfortunately, also in this case, similarly to what had happened during the first breeding cycle, the difficulties linked to the malfunction of the system and its relative technologies favoured the development

of saprolegnia, which caused the death of numerous eggs. In fact, the malfunctioning of the recirculation system, which should work via closed circuit with the biological filtration and sterilization of the water by means of UVA lamps, functioned with a continuous flow of water from outside, due to leakage that caused the tanks to empty and interrupted the recirculation. Moreover, the rise in temperature of the water in the breeding tank caused by the breakdown of the cooling system contributed to the development of an infection caused by a fungal pathogen. Fighting the saprolegnia, in a breeding environment can only be done by removing the dead eggs, which are more easily attacked by the fungus. This operation, in the case of the European bullhead, proved to be difficult, as the eggs were attached to the surface of the tiles or inside the perforated bricks, which are difficult to enter. Despite the development of the fungal infection, approximately 50% of the eggs hatched out and 500/600 larvae were born. After the reabsorption of the yolk sac, these were actively and abundantly fed with *Brine shrimp* nauplii. Approximately one month after hatching, the fry appeared to be very active, swimming freely along the column of water and showing a preference for the perforated bricks as shelter areas. The growth of the fry kept in the breeding farm was found to be in line with the biology of the species and was estimated to be approximately 1.5 cm one month after hatching and approximately 2.5 cm two months after hatching.

At the end of this *ex-situ* experimental cycle (2012), 469 European bullhead fry were released into the watercourse already identified as potential bodies of water in which to introduce the propagation material produced.

During the third reproductive season (2013), the procurement of breeding stock was more difficult due to complicated hydrological conditions of the watercourses, caused by heavy precipitations at the beginning of the year, with subsequent heavy flow rates and high speed of the currents. Nevertheless, the procurement of breeding stock, which continued on until the beginning of April, was adapted in relation to the conditions of the breeding tanks. In contrast to the previous year, there were no episodes of saprolegnia infection, probably thanks to the correct functioning of the cooling system, making it possible to maintain adequate control of the temperature inside the tanks (low water temperatures are unfavourable to attacks by fungal pathogens). Therefore, it was possible to improve the figure relative to hatching, which was estimated to be approximately 90% of eggs laid (500/600 newly born larvae), although the number of spawning events (4/5 nests) was lower than the previous year.

At the end of the third cycle of *ex-situ* experimentation, considering the length reached (approximately 20 mm), which was sufficient to guarantee survival once released into the natural environment, the propagation of the fry was brought forward by approximately two weeks, due to the fact that signs of the spread of saprolegnia were noticed in the two “hatchery” tanks. In total, 634 European bullhead fry were released into the watercourses that had already been identified as potential bodies of water in which the propagation material produced could be introduced.

In conclusion, once the problems linked to the correct functioning of the recirculation system had been solved, based on experience and by successfully controlling the quality and temperature of the water in the breeding farm, we believe that the figure relative to the number of spawning events and larvae produced using this protocol, which was perfected during this project, can be considered completely satisfactory, also in comparison to similar experiences carried out on the same species.

Particularly with regard to the housing of the breeding stock specimens, which were captured in a natural environment, the following considerations can be made:

1 – There was no occurrence of disease during experimentation;

2 – The behaviour exhibited by the species in a controlled environment is sociable and non-territorial;

3 – The breeding stock proved to be very adaptable as far as feeding is concerned;

4 – The gravel substrate was considered unnecessary. On the contrary, it threatened to penalize the cleaning and disinfecting system and was therefore eliminated;

5 – Perforated bricks can be used as shelters instead of tiles;
6 – High density during breeding is very well tolerated by the species.
As far as the reproduction and hatching phase is concerned, the following considerations apply:
1 – The technique used for hatching the eggs can now be considered standardized and consolidated;
2 – Ideal hygienic conditions need to be maintained and the development of saprolegnia needs to be avoided;
3 – Low mortality was observed after hatching;
4 – Growth proved to be rapid;
5 – There were no indications pointing to the need to use substrates or specific shelters for the young specimens.

Experimentation of a protocol for the breeding of the White-clawed crayfish

As expected, the first attempts at *ex-situ* breeding of the White-clawed crayfish brought uncertain outcomes, compared to the results from the breeding of the European bullhead. The Crayfish is a decidedly more sensitive species and has a somewhat complex reproductive biology, especially concerning the maturation of eggs contained in the egg sac and the weaning of larvae. The duration of the egg incubation period of this species is, in fact, rather long, involving the months from autumn (October-November) to the following spring (May-June). Similarly, the development of larvae, with the various shedding events that follow, involves several months and this makes the management of this species in the hatchery very challenging. To be added to this picture is the significant territoriality of the White-clawed crayfish, a characteristic that often leads to conflicts with lethal outcomes, especially in the delicate phase of shedding, when the specimens do not have a sufficiently resistant exoskeleton.
The procurement of A. pallipes specimens in the Apennine torrents, identified during preliminary studies, took place on 21 April and 4 June 2010. The specimens procured in nature were immediately transferred to the centre for experimental breeding of fish species at risk, operative at the Department of Evolutionary and Functional Biology of the University of Parma. Both in April and in June, 17 specimens of Crayfish were collected, making a total of 34. Among the Crayfish specimens captured, 5 of them had eggs in the abdominal area. Concurrently with the collection of the breeding stock, organic matter was also collected for genetic analysis. The egg clusters made it possible to obtain a limited number of larvae, which died during the days after hatching, again caused by an unexpected infestation of saprolegnia.
A decision was therefore made to begin again, with a new experimental programme, allowing for the arrival of the reproductive season 2010-2011 with specimens well-adapted to farming. In particular, the new protocol was tested using 5 female and 10 male survivors of diseases, cannibalism events and competitive interactions (the latter were decidedly pronounced during the initial periods of acclimatization in the tanks). Three of the five females proved to be reproductive and this made it possible to obtain 62 larvae from the eggs that hatched around mid-June 2011.
The larvae were fed with plant and animal foodstuffs (live and artificial foods), and the various shedding events were monitored, along with the rate of growth, up to the weaning of 21 specimens that reached sizes of 2.5-3 cm in the spring of 2012.
The experimental phase continued on until June 2012, when the specimens, which had reached 3.5 cm in size, were reintroduced into the natural environment, in the watercourses involved in the project.
Experimentation at the fish hatchery of Ponte San Giorgio (BO), which was created as part of the project, began in autumn 2012.
In the area of interest in the Province of Bologna, it was not possible to identify a large enough number of wild populations with a conservation status sufficient to be used as breeding stock destined for *ex-situ* breeding. For this reason, the breeding stock was procured from the Apennine torrents in the territory of

Prato, which is home to several populations of White-clawed crayfish that are viable and suitable for the purpose and, based on the outcome of the studies carried out in the territory of the Province of Prato, genetically compatible with the populations from the Bologna Apennine area.
This reproductive cycle, inside tanks with closed recirculation and conditions of controlled temperature, also proved to have different, more complex problems than in the artificial reproduction of the European bullhead.
At the end of October 2012, pairs of crayfish with behaviour interpreted as a reproductive ritual were noticed, while in November of the same year, females with egg sacs were detected. Unfortunately, at the end of November, the first problems arose. In fact, it was noticed that several females no longer had their eggs, which had probably been eaten by the females themselves. In order to identify any specific factors responsible for the loss of the egg masses, we decided to carry out chemical analysis of the water supply to the hatchery (from the torrent of Limentra di Treppio and the reservoir beneath) and of the waters in the tanks connected to the recirculation technology. The results from the analysis of the waters carried out at the Mario Negri Sud Institute (a contact activated following specific networking with the "CRAINAT" LIFE project) revealed high and potentially toxic concentrations of manganese and zinc in the tanks hosting the crayfish, while the water supply proved to be suitable for the species.
Overall, of the stock of females from the Prato territory that were put into the hatchery (no=14), only 7 produced eggs and these egg-laying females lost their eggs in different phases.
To remedy the high concentrations of zinc and manganese (toxic for the species) found in the tanks and probably caused by the technology used, a new line dedicated to the breeding of the Crayfish was set up, created using 3 rectangular trough-type tanks, stacked using a metal structure, functioning via an open circuit and with a continuous flow of water from the breeding farm's water supply well. In April 2013, outings to watercourses external to the project area took place, with the aim of searching for new quotas of egg-laying females to be transported to the breeding farm. Only two egg-laying females were procured, which is a very limited number, but still usable for completing the experimentation of a new breeding cycle and, at the same time, protecting the already diminished populations present in the territory.
At the end of the May, part of the eggs (approximately 50%) of both of the freshly captured egg-laying females appeared to be hatched and the newly born larvae were attached beneath the abdomen. Approximately 40 crayfish were born from the two newly captured females and at the end of June 2013 and their lengths were estimated to be approximately 10/13 mm. The young were fed with pellets made from finely ground vegetal flour. Their growth was verified, based on bibliographical data, and once they had reached a length of 2.5 cm, which occurred in the first two weeks of September 2013, the 33 surviving specimens and the breeding stock were reintroduced into nature.
In conclusion, the experiments conducted on the *ex-situ* reproduction of the White-clawed crayfish demonstrated that breeding stock can also be procured late in the reproductive season (April-May) using specific campaigns aimed at their capture, thus avoiding long periods in the breeding farm that would expose the egg-laying females to risks, like variations in the chemistry of the waters, the attack of pathogens or even territorial disputes, which often take place in controlled, restricted environments imposed by artificial breeding. Proceeding in this way makes it possible to focus on improving the efficiency of hatching.

IN-SITU REPRODUCTION OF THE EUROPEAN BULLHEAD AND WHITE CLAWED CRAYFISH

The improvement of the conservation status of the populations of *A. pallipes* and *C. gobio* also involved developing an operating protocol to encourage *in-situ* reproduction (i.e. within the natural habitat) in the two species.
In order to recover the populations which have become rare and encourage the reintroduction of seeded specimens, from farming attempts, improvements have been made by restoring the natural substrates and introducing artificial micro-habitats in certain rivers within the territory involved in the project.
At the start of the study, no scientifically accepted experimental data existed on the use of artificial structures for *in-situ* reproduction; it was therefore a question of conducting a scientific experiment using micro-habitats, and at the same time counteracting the competitors present in the environment. In fact, the preliminary studies highlighted the possibility of disturbance and/or competition for the indigenous populations of both *A. pallipes* and *C. gobio* from the brown trout, and we therefore temporarily suspended trout seeding in some specific areas of the rivers where the experiment was conducted.
The experiment was based on the results obtained from the fish censuses performed during the preliminary studies, which provided a clear picture of the size, in terms of biomass, density and structure of the wild populations in the two target species. The experimental part was launched in the first quarter of 2011, in order to include the reproduction period of *C. gobio* (running from the end of February to the first half of April) and get a better chance of repeating the monitoring and evaluating the actual effectiveness of the micro-habitats created. This also allowed us the chance to compare the status of the populations before and after creating the fish ladders in the summer of 2011.

Choosing the sites

The first step in the project was to choose the areas in which to operate. This choice was made according to the presence, size and above all structure of the population in the various areas covered by the census, as well as the areas' suitability for reproduction purposes.
Considering the convergence of the goals, we decided to use the same areas for both *in-situ* reproduction and for the fish ladders, as well as for the *ex-situ* repopulation activities in general.
This has led to the identification of two areas in the Prato area which are suitable for promoting *in-situ* reproduction of *C. gobio*: the upper course of the River Bisenzio in the Molino della Sega area, and the lower part of the Rio Ceppeta, just upstream of the convergence with the River Bisenzio.
For *A. pallipes*, on the other hand, we decided to intervene in one area of the Rio Ceppeta.
These sites were in addition to two other sites on the Brasimone torrent, in the territory of Bologna: in this case, the choice was based on the indication of experts at the Lakes Suviana and Brasimone Natural Park.

The experiment conducted

Given that we are working with very natural environments, it may seem superfluous to create artificial mating sites for the two species in question. Our first priority was to encourage *in-situ* reproduction as far as possible by limiting predators and using small habitats located on the riverbed in order to guarantee the right conditions for laying eggs and the early larval stages of development in general.
In addition to this, in February 2'11 we placed some small semi-natural or completely artificial structures designed for the purpose on the riverbed. These structures (tiles, perforated bricks, air bricks, bundles of tubes, elements for artificial weaning), selected according to the species to be promoted, were placed in the 7 stations identified: Rio Ceppeta - Station CE_01; Rio Ceppeta - Station CE_02; Rio Ceppeta - Station CE_03; Trogola-Alto Bisenzio - Station TR_01; Trogola-Alto Bisenzio - Station TR_02; Brasimone torrent -

Station BR_01; Brasimone torrent - Station BR_02.
Activities aimed at improving *in-situ* conservation for *A. pallipes* were concentrated in the Rio Ceppeta, laying air bricks and perforated bricks along with tube bundles for European bullheads.
On the Trogola-Alto Bisenzio torrent and the Rio Ceppeta at Mulino della Sega up and downstream of the two dams present, we placed tiles, perforated bricks and tube bundles; at a later date, exclusively on the Trogola-Alto Bisenzio downstream of the dam, we also added some artificial weaning elements.
On the Brasimone torrent, given the presence of both target species, we decided to introduce *in-situ* all the available types of substrate: tiles, perforated bricks, air bricks and tube bundles.
We also performed manual morpho-functional interventions in all the survey sites, with the aim of encouraging the reproductive processes of the two species.
After placing the semi-natural or artificial structures on the riverbed in February 2011, we conducted regular inspections of the sites during the mating seasons of the two target species (April, May and September 2011, April, May and October 2012), both in order to check if specimens from the two target species were actually colonising the areas (or the presence of eggs laid by *C. gobio*) and to clean and repair the substrates if necessary.

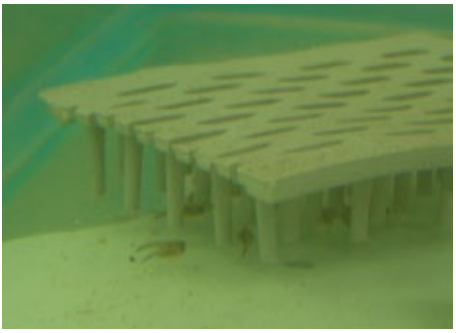
Apart from modelling the riverbed and placing the artificial structures, we also took action to counteract potential predators (salmonid fauna and eels).
Regarding competition with humans:

- In general, *C. gobio* is no longer fished for human consumption, apart from in certain Alpine and Pre-Alpine areas, where it is still appreciated and used for typical local dishes. It is also sometimes used as bait for trout fishing;
- Fishing activities is no longer a direct threat to *C. gobio*. Nonetheless, the management of fish stocks in water courses, with the repopulation of predators and use as bait, has affected the structure and size of the population. Some authors81 have hypothesised that the repopulation of salmonidae and the destruction of the habitat have had a greater impact on *C. gobio* than water pollution.

For this reason, in collaboration with the Province of Prato Hunting and Fishing Service, we have drawn up two separate Action Plans (approved by Provincial Decree 43/2012) for the conservation of the respective target species, aiming to eliminate seed banks in the areas identified for the experiment and strongly limiting those nearby, using only young stock.
Regarding the goal of reducing competitors, the conservation action plan for *C. gobio* specifically states the following:
- Within the Prato Apennine SIC, the practice of seeding adult Salmonidae should be prohibited in order to permit the correct coexistence between the fish populations;
- The practice of repopulation using young brown trout [*Salmo (trutta) trutta*] from the Mediterranean stock (fry or at the most 4-6cm trout fingerling, with a density of 0.05 specimens/m2 of around 500 ind/ha), possibly self-producing in incubators downstream of the native reproducers taken from provincial bodies of water, would have a lower impact and therefore fit in better with the European bullhead conservation goals;
- "We also propose prohibiting repopulation with juvenile brown trout stock in the areas where the environmental protection and improvement activities will be taking place along the Rio Ceppeta a Cantagallo, Rio Ceppeta and Rio Trogola at Molino della Sega, and limiting them to the strictly necessary in other areas. These prohibitions or regulations should also apply to the surrounding areas which may directly affect the European bullhead populations within the SIC".

A similar scheme to limit competitors was included in the conservation Action Plan for *A. pallipes*:
- "We propose preventing repopulation initiatives using juvenile salmonidae stock along the Rio Ceppeta

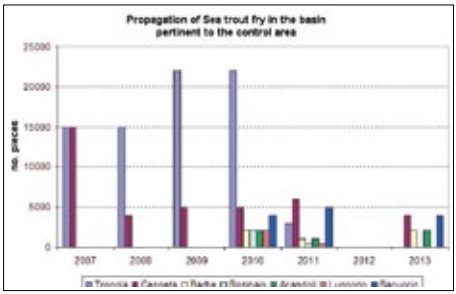


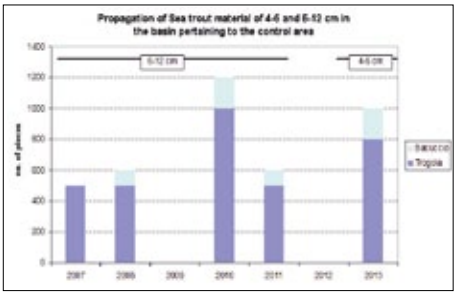
Figg. 69, 70, 71, 72 – Some of the artificial substrates used: in order, tiles, air bricks, perforated bricks, artificial weaning elements.

and the areas where the environmental protection and improvement activities will be taking place, as well as limiting them to a minimum in other bodies of water within the SIC (fry or at the most, 4-6 cm trout fingerlings with a density of 0.05 specimens/m2 equal to approx. 500 ind/ha);
- The practice of seeding adult fish should be prohibited within the Prato Apennine SIC area, in order to allow the native fish populations and the white-clawed crayfish to establish a correct coexistence;
- Seeding initiatives should certainly be avoided in the areas (and periods) dedicated to reproduction, incubation and hatching, i.e. from November to June".

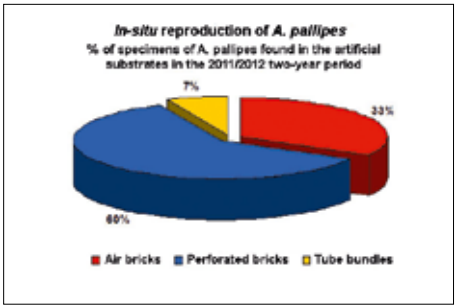
Tab. 5 shows how after 2012, when the measures set out in the conservation Action Plans for the target species came into effect, seeding with fry fell drastically, and in 2013 were limited to the lower basin of the Trogola stream, the Rio Barba and the Rio Acadoli, which are located a long way upstream from the areas of interest. Regarding the lower basin of the Rio Ceppeta, the seedings that took place in 2013 only covered high altitude parts of the Rio Ceppeta and its affluent Bacuccio: these areas were also chosen at a due distance from the areas where the experiments were being conducted to encourage *in-situ* reproduction.
Regarding brown trout juveniles measuring between 4 and 12 cm, as shown in Tab. 6, no seedings took place in 2012, while in 2012 1,000 fingerlings measuring 4-6 cm were seeded, most of which were introduced to the higher course of the Bisenzio, downstream of the experiment area and along a small part of the Rio Bacuccio.
The evaluations set out above show that after 2012, no adult direct competitors for the target species



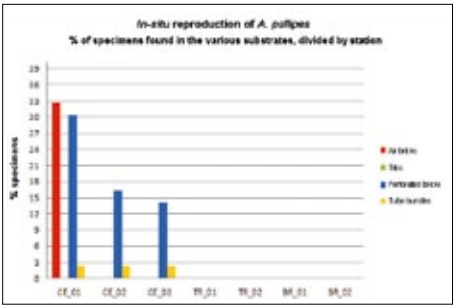
Tab 5 – Brown trout fry seedings (2007-2013) in the bodies of water surrounding the control area.



Tab. 6 – Brown trout fingerling seedings (2007-2013) aggregated by size, in the water bodies surrounding the control area.



Tab. 7 – Shows the percentage of *A. pallipes* specimens found in the various substrates during the 2011/2012 two-year period.



Tab 8 – % efficiency of the artificial substrates for *A. pallipes* divided by site.

were introduced to the area directly affected by the experiment. Regarding the trout fingerlings, their use as seeding material was limited to 4-6 cm forms, introduced in small quantities and not directly in the experiment sites. Lastly, the quantities of fry were considerably reduced.

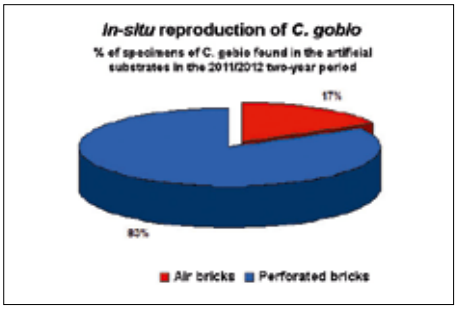
As shown in the diagram above, the perforated bricks were the most popular substrates for the decapod, containing 60% of the specimens found.

The air bricks also appeared to function well, considering that they were used in 3 sites out of 7, and that one of these sites alone, the Rio Ceppeta station, accounted for 33% of the total, corresponding to 14 specimens overall (see Tab. 8).

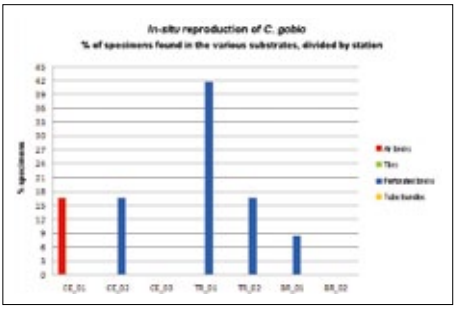
On the other hand, the PVC tubes had a very low success rate (7%), showing that although they may be an excellent substrate for reproduction and breeding species in captivity, they are not suitable for *in-situ* reproduction within this specific environmental setting.

The *in-situ* activities for *A. pallipes* at the Rio Ceppeta CE_01 station met with huge success in comparison with the other stations; in fact, the diagram above shows how 65% of the total number of crayfish found in the 2011/2012 period was attributed to this station. The substrates up and downstream

Fig. 73 – *A. pallipes* specimen found inside a perforated brick.



Tab. 9 – % efficiency of the artificial substrates for *C. gobio.*



Tab. 10 – % efficiency of the artificial substrates for *C. gobio* divided by site.

from the Rio Ceppeta dam (CE_02 and CE_03) were considerably less successful, with percentages of 19% and 16% respectively.

Regarding *C. gobio*, Tab. 9 below shows the percentage of specimens found in the various substrates during the 2011/2012 two-year period. Just like we observed for the white-clawed crayfish, the perforated bricks appeared to be the most suitable substrates, accounting for 83% of the total specimens found.

The air bricks on the other hand had a decidedly lower success rate, accounting for the remaining 17%, and only in the Rio Ceppeta CE_01 station (Tab. 10). The tiles had an insignificant rate of success.

Overall, the results obtained by the experimentation with this species, especially in comparison to those with *A. pallipes*, were somewhat scarce in terms of total numbers. In fact, we found only 12 specimens in the substrates, 42% of which in the Trogola-Alto Bisenzio area upstream of the dam.

The interpretation of the results, if associated with the European bullhead population trends resulting from quantity monitoring, leads us to believe that the species clearly prefers natural substrates and cracks to artificial substrates as refuges and/or laying sites.

To summarise, we could therefore conclude that:

- The perforated bricks were the artificial substrate preferred by *C. gobio*;
- The air bricks, followed by the perforated bricks, were the artificial substrates preferred by *A. pallipes, considering the fact that air bricks were used only in CE_01 station*;
- The substrates used were also frequented by the two species during the reproductive period;

Fig. 74 – *C. gobio* eggs laid, found during manual sampling.

Fig. 75 – *C. gobio* specimen found inside a perforated brick.

- There was no correlation between the number of specimens found inside the substrates and the biomass or density estimated through quantity sampling;
- the combined effect of all the operations conducted to encourage the target species (creating fish ladders, natural habitats, using artificial substrates and limiting predators) led to a clear success for the populations of both target species, as shown in more detail in the results of the biological monitoring (see the relative paragraph below).

Restoring water continuity

The creation of the fish ladders allowed us to restore water continuity between two water courses in the Apennines (the Trogola-Alto Bisenzio torrent and the Rio Ceppeta), previously interrupted by two dams constructed not for hydro-geological stability, but in order to allow the waters from these two streams to be deviated towards the "Mulino della Sega" water mill (in the past, the flow of water was used not only to mill, but also to power an hydraulic saw). The photo below shows the dam on the Trogola-Alto Bisenzio before our intervention.
The work, outsourced by tender, was begun on 29 July 2011 and completed on 24 September 2011.
Strong populations of *C. gobio* were found in both water courses (Bisenzio and Ceppeta): the type of fish steps chosen was designed with special features to encourage this particular target species. In fact, sharp, 15-20 cm large stones have been inserted at the bottom of the ladder and sunk into the cement, specifically in order to create the micro-topographical conditions (rugged riverbed) that are particularly suited to the European bullhead's movement, which moves in short jerks, exploiting the resting places (cobbles or shingles) along the way (see photos below).

Fig. 76 – Dam on the Trogola-Alto Bisenzio before the intervention.

Figg. 77, 78 – Detail of the bottom of the ladder and image of a *C.gobio* specimen on the ladder.

Fig. 79 – Floodgates opening at the bottom to allow water passage.

The flow of water, when particularly high, is slowed by the presence of floodgates opening at the bottom only on one side (see photo below), to make it easier even for fish species with scarce agility, such as the Bullhead, to climb the ladder.
The works were protected using fully walkable metallic grates, to protect them from the flooding, predatory birds, illegal fishing and avoid accidental falls. This grating is removable to allow for maintenance and monitoring activities.

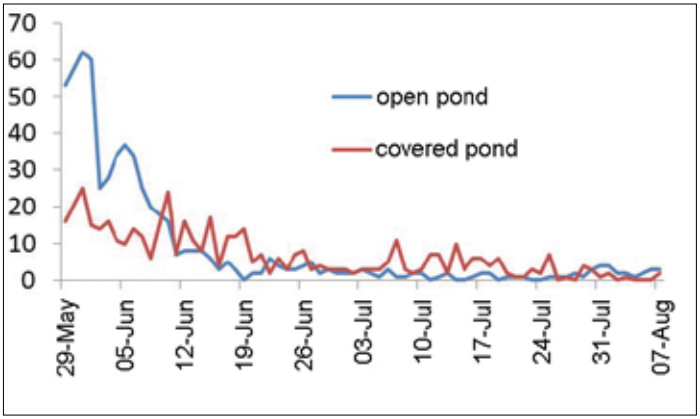
The photos below show the completed works along the Trogola-Alto Bisenzio (first photo) and the Rio Ceppeta (second photo), fitting in well with the surrounding environment.

Figg. 80, 81 – Dams constructed along the Trogola-Alto Bisenzio and Rio Ceppeta respectively.

Experimentation of methods of controlling invasive alien species

The preliminary studies and periodic monitoring performed in the wetland areas of the Prato plains highlighted the negative impact on the conservation status of the target species of the presence of various invasive alien species who are natural competitors, as already described in an earlier paragraph of this volume.
In the spring of 2013, we therefore launched an experimentation of possible methods for controlling the populations of these species in the environments affected by the redevelopment as part of the "Water SCIs" LIFE project: site 01 Lake Pantanelle, site 02 Lake Bogaia and site 03 Lake Ombrone.
In site 01 Lake Pantanelle, we performed initiatives to control the Louisiana crayfish (*Procambarus clarkii*) and Bullfrog (*Lithobates catesbeianus*) populations, as well as limiting the expansion of a bamboo patch (*Phyllostachys spp.)* present in a small area at the lake's bank.

Fig. 82 – Captured Louisiana crayfish specimen.



Tab. 11 – Capture frequency trend.

In order to catch the crayfish and bullfrogs, we used 12 double-entry fish traps positioned in various points of the wet area, in particular in the hatchery-ponds for amphibians, where we found numerous specimens of *P. Clarkii*. The fish traps were baited with trophic bait to make them more effective, and we monitored them on a daily basis from 29 May to 7 August 2013.

We captured a total of 2 specimens of *L. catesbeianus* AND 972 specimens of *P. clarkii*, with a considerable reduction in the frequency of capture over time, which passed from more than 60 crayfish a day in the initial period to only a few towards the end of the experimentation, when the hatchery-ponds (fed exclusively by rainwater) and pools where the fish traps were placed reached their minimum annual water level.

We limited the expansion of the bamboo (*Phyllostachys spp.*) by pruning with billhooks and painting with a mixture of water-based 50% glyphosate or triclopyr herbicide. A series of small sized saplings (covering around 15 m2) were spray treated with a water-based 10% glyphosate solution. The cut plants were left at the site.

Overall, we treated 394 specimens: 338 with the glyphosate solution, and 56 with the triclopyr solution. The work was done on two occasions: 8 August and 6 September 2013.

In site 02 Lake Bogaia we eliminated 35 black locust trees (*Robinia pseudoacacia*) present in the area surrounding the wetland bog area. The intervention took place on 8 August 2013, using two different intervention techniques: “cut-inject” and “cut-paint”, using a total of 600 ml of 50% water and glyphosate solution. The resulting waste material was left at the site.

Fig. 83 – Reducing the population of *Phyllostachys spp.*

Fig. 84 – Intervention to control *Robinia pseudoacacia.*

Fig. 85 – Sun-deck trap used in site 02 Lake Bogaia.

In particular, the "cut-paint" method was used on 30 plants with a diameter of less than 10 cm, while the cut-inject method was used for the remaining 5 plants with a diameter of over 10 cm.

Still in the same wetland area of Bogaia, where we found at least 12 specimens of Pond slider (*Trachemys scripta*) in a visual survey, in the period between 3 October and 6 November we positioned two sun-deck traps, specially designed to catch turtles.

This type of floating trap take advantage of the turtles' need to leave the water to "bask": as cold-blooded animals, they need to regulate their body temperature using the heat of the sun. The sun-deck trap used (see Fig. 085) is formed of a floating structure made out of PVC tubes, fitted with a net placed under the PVC structure and fastened on the four sides of the quadrilateral. Above the structure, a wooden walkway and ramp encourages turtles to climb up the outside of the trap.

Turtles are caught when they decide to dive back into the water, and get trapped in the netting attached inside the PVC quadrilateral. Compared to other types of trap, sun-deck traps have the advantage of allowing the captured specimen to survive, and are also more selective than other methods, considerably

Fig. 86 – Action to control Bohemian knotweed.

reducing the possible negative impact on other species of animals present.
However, using the traps did not lead to the desired result, since they were repeatedly tampered with (dragged to the banks) by unknown persons during the period chosen for the experiment, clearly stopping them from working.
Lastly, at site 03 Lake Ombrone, we experimented with four different techniques of controlling Bohemian knotweed (*Reynoutria x bohemica*).
The experiment design involved applying the following containment methods in four 5x5m. plots:
1 – Applying a 10% glyphosate herbicide, repeated the following month;
2 – Applying a 10% glyphosate herbicide, followed by cutting the weeds on two occasions;
3 – An initial cut-back, followed by two applications of 10% glyphosate herbicide;
4 – Three repeated cut-backs.

The first three treatments described all had positive results, with a drastic reduction of the invasive alien species, while we noted that in the plots only subjected to mechanical cut-backs, not only was the knotweed reduced, but there was also a clear change in the vegetation present, with the growth of numerous nitrophilous species.
The interventions took place in the period from 8 August to 26 November 2013.

MONITORING THE TARGET SPECIES

The fauna censuses to ascertain the state of conservation in the target species were conducted using the methods set out in the monitoring protocol (available to view and download from the project internet site) developed during the preparatory phase in order to evaluate the effects of the active conservation measures taken.
The frequency of the monitoring depended on the biological requirements and phenology of the target species, avoiding subjecting them to excessive population stress, considering, for example, that the most common technique used and scientifically consolidated for censuses of fish and astacidae species involves capturing specimens, measuring their physical parameters and subsequently releasing them.
The stations chosen for monitoring *C. gobio* and *A. pallipes* were on the Trogola-Alto Bisenzio and Ceppeta streams in the province of Prato, and the Brasimone stream in the province of Bologna: in one research station on the Rio Ceppeta, we measured the effectiveness of natural and artificial micro-habitats for the spontaneous reproduction of *A. pallipes*. On the same water course, and on the upper course of the Bisenzio, we evaluated the effectiveness of natural and artificial micro-habitats for the spontaneous reproduction of *C. gobio* and the functionality of the fish ladders.
On the Brasimone stream, we evaluated the effectiveness of the micro-habitats for the spontaneous reproduction of both target species.
Furthermore, the scientific consultants of the Lakes Suviana and Brasimone Natural Park conducted specific monitoring activities to evaluate the effectiveness of the conservation measures taken along the upper areas of the main water courses in the territory of the Province of Bologna, where the initiatives aiming to reintroduce the two target species were focussed, including some of the minor affluents of the Limentra di Treppio stream (Rio Casale, Rio delle Fabbriche and Rio del Bago) given that, although outside the perimeter of the "Laghi di Suviana e Brasimone" SCI, crayfish have been found in these locations in the past.
Overall, the monitoring and census activities were performed according to the following calendar, indicating, for each site covered, the identification number (ID), name of the water course in questions, a reference place name, the altitude and the type of census conducted (quantitative or semi-quantitative).

Toponym	Altitude	2011					2012					2013			
	(m above sea level)	-5apr	-23may	-19sep	-22nov	-22dec	-20jun	-21jun	-19sep	-20sep	-10oct	-7jun	-18jun	-18sep	-15oct
Le Fabbriche Vecchie	540					Sq		Sq		Sq			Sq	Sq	
Ponte San Giorgio	480	Q		Q			Q		Q		Q	Q			Q
Molino dei Sassi	535				Sq			Sq		Sq			Sq	Sq	
Cà di Romicca	505				Sq			Sq		Sq			Sq	Sq	
Mangiamele	860		Q	Q				Q	Q		Q	Q			Q
Lavaccioni di Sotto	920								Sq			Sq			

Table 12 – Province of Bologna - Monitoring stations and periodicity (Q=quantitative survey, Sq=semi-quantitative survey).

The censuses of fauna and birds conducted after the preliminary studies were performed every two weeks from the autumn of 2010, in the areas affected by the environmental improvement actions part of the "Water SCIs" LIFE project: Lake Pantanelle (site 01), Lake Bogaia (site 02) and Lake Ombrone (site 03).
Lastly, the herpetological monitoring (amphibian species) followed the frequency indicated in the table below, reiterated in 2011-2013, following the first monitoring campaign (preliminary studies) performed in 2010:

Months	Number of times repeated
February	1
March	2
April	2
May	2
June	1

Table 13 – Frequency of herpetological monitoring.

Biological monitoring of ichthyofauna and astacofauna

Monitoring the European bullhead

The experimentation with *C. gobio* was conducted in 4 different stations in the province of Prato and 2 in the province of Bologna, identified through a careful *pre-survey*. We tried to focus the checks of the various operations performed on the same sites, already identified as suitable, including:

- *In-situ* activities designed to encourage the species' suitability by using both naturalistic-environmental habitats and laying different materials (artificial substrates);
- *Ex-situ* activities, involving genetic profiling and sampling selected reproducers, egg-laying, weaning juvenile material and subsequent seeding;

The construction of 2 fish ladders and verification of their functionality.

Overall, the results were very positive, and we found a general improvement in the size of the European bullhead population in all the stations monitored. Only the findings for 2013 showed a fall, although remaining well above the estimated biomass and density before the beginning of the project.
This fact was easily explained by the weather events that took place in March 2013: in Tuscany, some exceptional weather events took place, with average rainfall of around 250 mm and peaks of over 900 mm (in the Province of Lucca).
In the entire regional territory, average rainfall was higher than the average period of reference (1983-2012) with a surplus value of around 100-150%. The persistence of the rain caused several hydro-geological landslides and the flooding of the Ombrone and the Bisenzio in the Pistoia area. This event also affected the area of study, as is clear from the image below, reaching cumulative values of 80-100 mm (Fig. 87).
Events of this kind, occurring in a limited period of time, clearly have a considerable negative impact not only in hydro-geological terms, but also on the biota of the ecosystem. In the case in question, the effects were particularly marked, given that *C. gobio* is a typically benthic fish species (i.e. it mainly lives on the bottom of water courses). The analysis of the data divided by individual cohorts clearly shows how the loss of biomass and density were not legible in the 0+ cohort, but only on the level of more adult specimens. This is because the event, which also temporarily compromised the functionality of the fish ladders, took place before the eggs were laid, and therefore had very little effect on the 0+ cohort, which was effectively saved from the event.
The following tables show the comparative results for each of the stations monitored, with a brief comment on each.

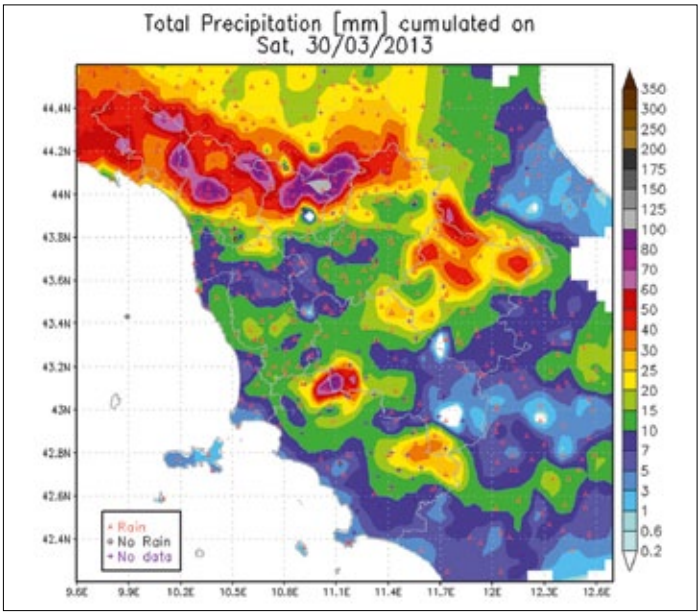
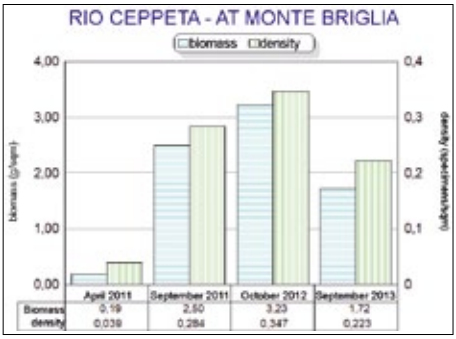


Fig. 87 – Accumulated rainfall on 30 March 2013 – maximums over 100 mm in Northern Tuscany and the Pistoia/Prato Apennines.

Rio Ceppeta - Station CE_02

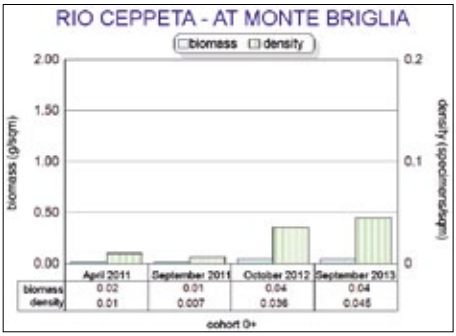
Scientific name	Date	B (g/m²)	D (specimens/m²)	A.I.	Structure
Cottus gobio	06/04/2011	0.19	0.039	3	1
	14/09/2011	2.50	0.284	4	4
	01/10/2012	3.23	0.347	5	1
	13/09/2013	1.72	0.223	4	1

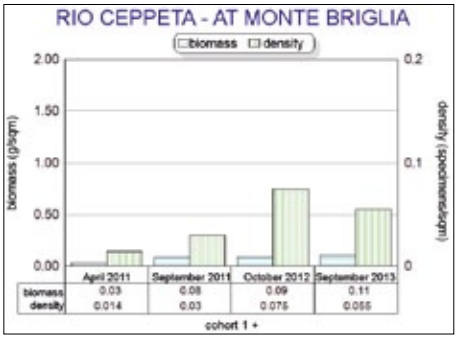
Tab. 14 – Biomass and density of *C. gobio* in CE_02 in the 2011-2013 period.

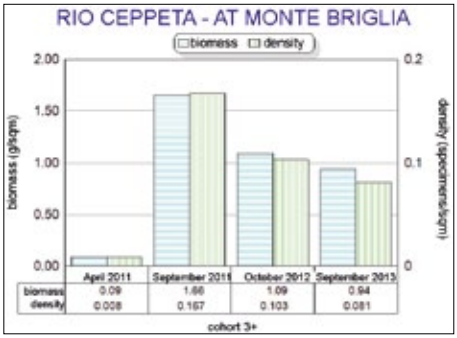


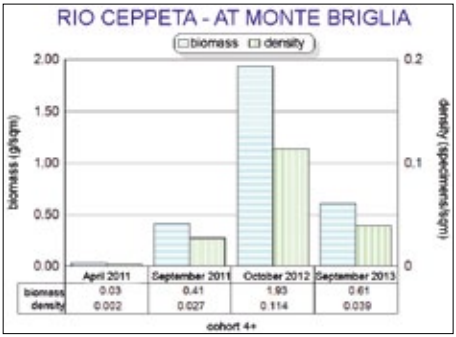
Tab. 15 – Biomass and density values for C. gobio estimated during quantitative monitoring conducted in the 2011-2013 three year period.

The age classes present range from 0+ to 4+; the diagrams below show the estimated biomass and density values for the European bullhead over the years, divided by cohort.









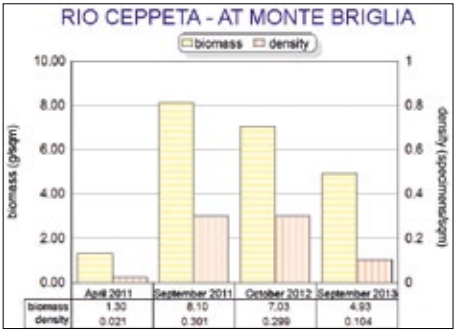
Tables 16a, b, c, d, e – Biomass and density by age class in *C. gobio* in CE_02.

We also present an analysis of the data on potential predators - in this specific case, the brown trout: the tables below summarise the data on estimated density and biomass for the brown trout during the monitoring activities conducted in the 2011-2013 three year period.

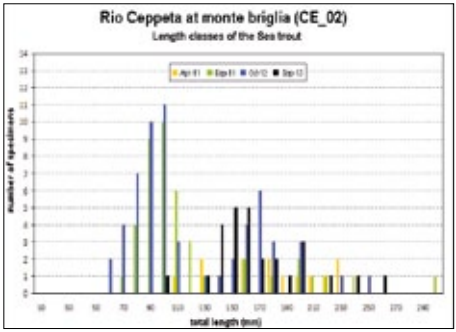
Scientific name	Date	B (g/m²)	D (specimens/m²)	A.I.	Structure
Salmo (trutta) trutta	06/04/2011	1.30	0.021	2	4
	14/09/2011	8.10	0.301	4	1
	01/10/2012	7.03	0.299	5	1
	13/09/2013	4.93	0.104	4	1

Tab. 17 – Biomass and density in S. (trutta) trutta in CE_02 in the 2011-2013 period.

The diagram shows that both parameters rose sharply between April and September 2011; in fact, biomass and density increased from 1.30 g/m² and 0.021 ind/m² to 8.10 g/m² and 0.301 ind/m² respectively. The trends for both parameters continued to rise constantly from then onwards, in line with expectations, especially in the more recent years after the application of the measures proposed in the action plan, which considerably reduced fish seeding activities.
The phenomenon is even clearer in the figure below, showing the trend by length class. However, given that the fishery activities are being conducted in an environment where it not possible to remove specimens, it is clear that it will take several years to achieve a new balance.



Tab. 18 – Biomass and density values in S. (trutta) trutta estimated during quantitative monitoring conducted in the 2011/2013 three year period.

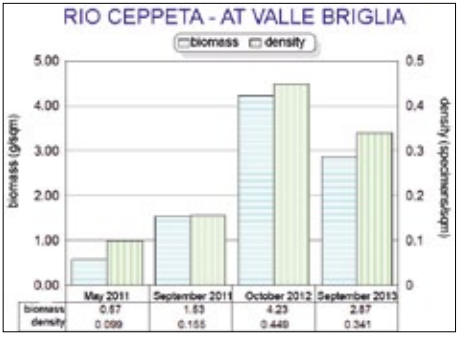


Tab. 19 – Distribution in length classes of S. (trutta) trutta in the 2011-2013 period.

Rio Ceppeta - Station CE_03

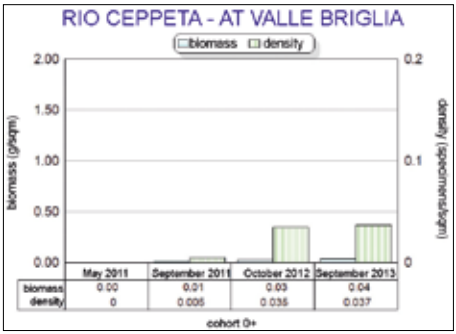
Scientific name	Date	B (g/m²)	D (specimens /m²)	A.I.	Structure
Cottus gobio	17/05/2011	0.57	0.099	3	4
	14/09/2011	1.53	0.155	4	4
	03/10/2012	4.23	0.449	5	1
	12/09/2013	2.87	0.341	5	1

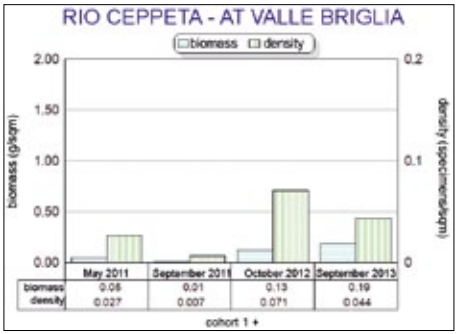
Tab. 20 – Biomass and density of *C. gobio* in CE_03 in the 2011-2013 period.

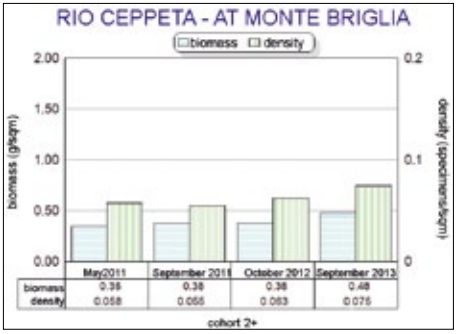


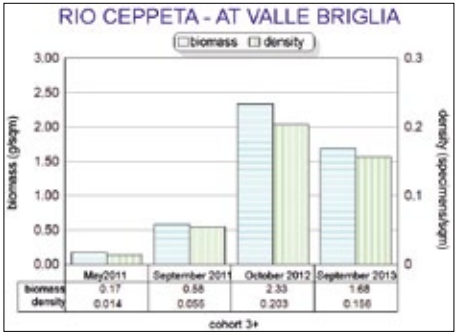
Tab. 21 – Biomass and density values for *C. gobio* estimated during quantitative monitoring conducted in the 2011-2013 three year period.

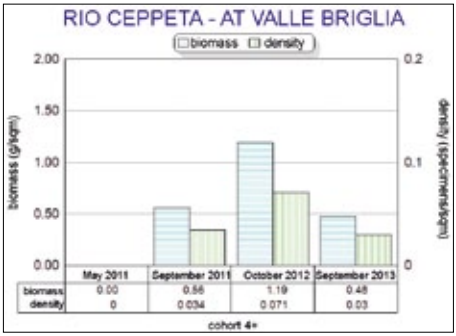
Both parameters rose, achieving their maximum value in the month of October 2012, with a density of 0.449 ind/m² and a biomass of 4.23 g/m²; the species, according to the semi quantitative abundance indicator, can be defied as dominant and with a well-structured population in the area where samples were taken.
The age classes present range from 0+ to 5+; the diagrams below show the estimated biomass and density values for the European bullhead over the years, divided by cohort.

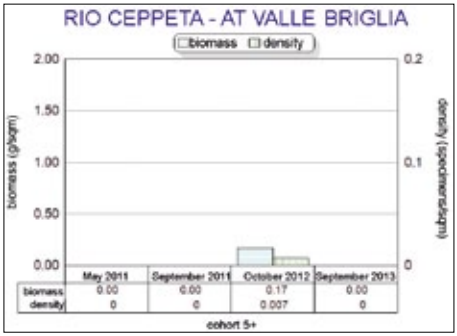










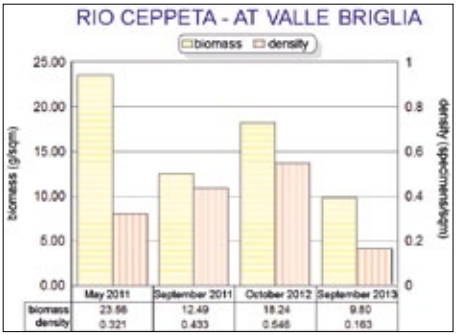


Tab. 22a, b, c, d, e, f - Biomass and density by class in *C. gobio* in CE_03

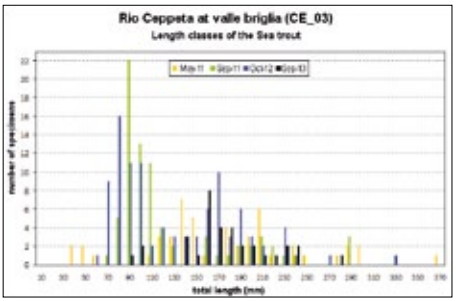
The following tables summarise the data on estimated density and biomass for the brown trout during the monitoring activities conducted in the 2011-2013 three year period.

Scientific name	Date	B (g/m²)	D (specimens/m²)	A.I.	Structure
Salmo (trutta) trutta	17/05/2011	23,56	0,321	5	1
	14/09/2011	12,49	0,433	5	1
	03/10/2012	18,24	0,546	3	1
	12/09/2013	9,80	0,163	4	1

Tab. 23 – Biomass and density in S. (trutta) trutta in CE_03 in the 2011-2013 period.



Tab. 24 – Biomass and density values in S. (trutta) trutta estimated during quantitative monitoring conducted in the 2011-2013 three year period.



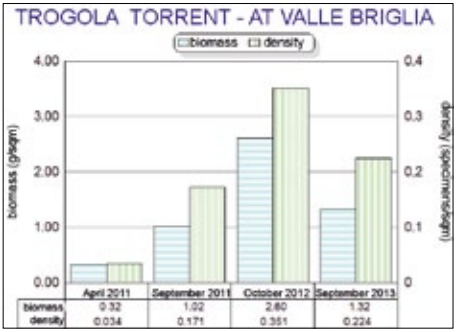
Tab. 25 – Distribution in length classes of S. (trutta) trutta in the 2011-2013 period.

Here too, the brown trout population fell noticeably.

Trogola-Alto Bisenzio - Station TR_01

Scientific name	Date	B (g/m²)	D (specimens/m²)	A.I.	Structure
Cottus gobio	06/04/2011	0.32	0.034	3	2
	14/09/2011	1.02	0.171	4	1
	02/10/2012	2.60	0.351	5	1
	13/09/2013	1.32	0.224	5	1

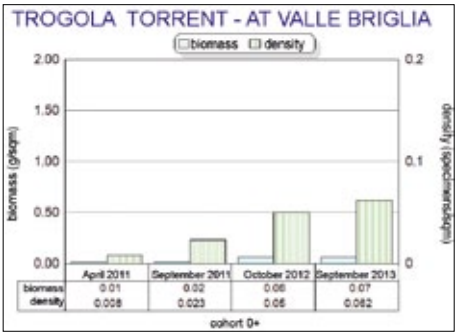
Tab. 26 – Biomass and density in *C. gobio* in TR_01 during the 2011-2013 period.

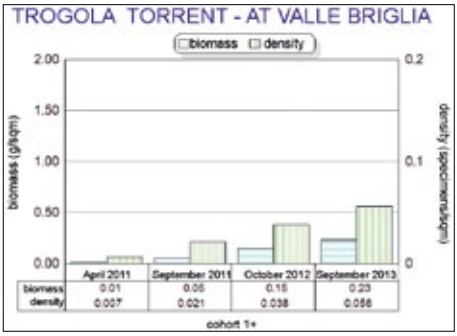


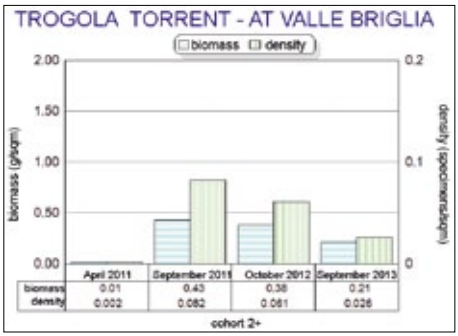
Tab. 27 – Biomass and density values for *C. gobio* estimated during quantitative monitoring conducted in the 2011-2013 three year period.

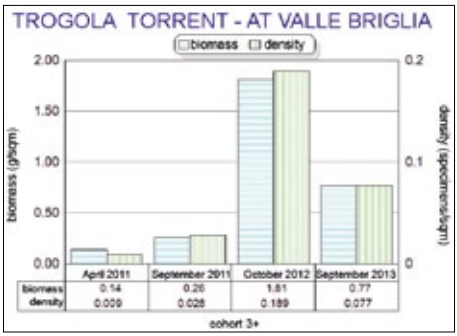
Just as observed in the Rio Ceppeta, both the biomass and density showed a growing trend, which reached its maximum value in October 2012, with 2.60 g/m² and 0.351 ind/m² respectively; the semi-quantitative abundance indicator attributed the species a value of 5, demonstrating its dominance in the area sampled. The population appears structured. The same observations made for 2013 also apply here, including the impact of the exceptional weather event.

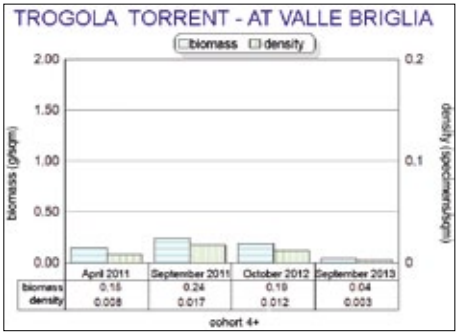
The age classes present range from 0+ to 4+; the diagrams below show the estimated biomass and density values for the European bullhead over the years, divided by cohort.









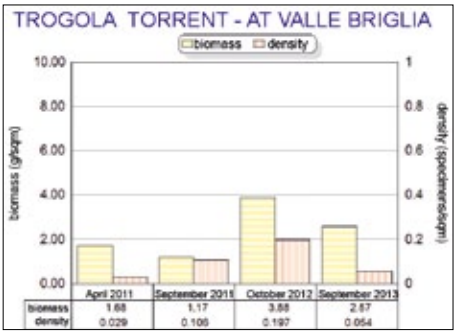


Tab. 28a, b, c, d, e – Biomass and density by age class in *C. gobio* in TR_01.

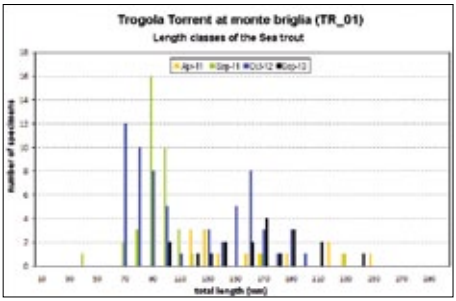
The following tables summarise the data on estimated density and biomass for the brown trout during the monitoring activities conducted in the 2011-2013 three year period.

Scientific name	Date	B (g/m²)	D (specimens/m²)	A.I.	Structure
Salmo (trutta) trutta	06/04/2011	1.68	0.029	3	4
	14/09/2011	1.17	0.106	4	1
	02/10/2012	3.88	0.197	5	1
	13/09/2013	2.57	0.054	2	4

Tab. 29 – Biomass and density in S. (trutta) trutta in TR_01 in the 2011-2013 period.



Tab. 30 – Biomass and density values in S. (trutta) trutta estimated during quantitative monitoring conducted in the 2011-2013 three year period.



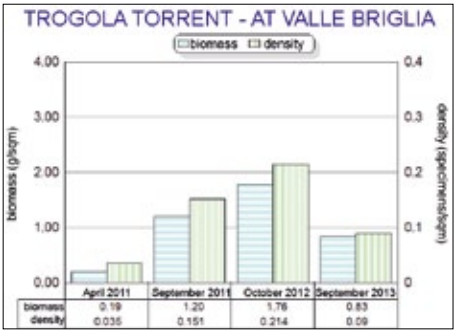
Tab. 31 – Distribution in length classes of S. (trutta) trutta in the 2011-2013 period.

Once again, the diagram using length classes clearly shows a fall in the population.

Trogola-Alto Bisenzio - Station TR_02

Scientific name	Date	B (g/m²)	D (specimens/m²)	A.I.	Structure
Cottus gobio	21/04/2010	0.19	0.035	3	1
	15/09/2011	1.20	0.151	5	1
	02/10/2012	1.76	0.214	5	1
	12/09/2013	0.83	0.090	4	1

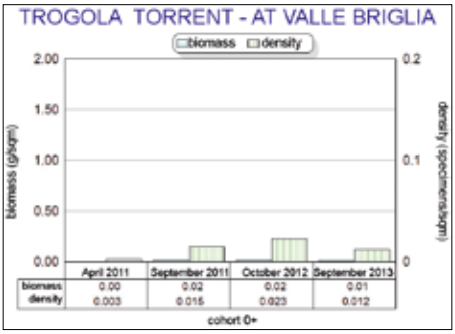
Tab. 32 – Biomass and density in *C. gobio* in TR_02 in the 2011-2013 period.

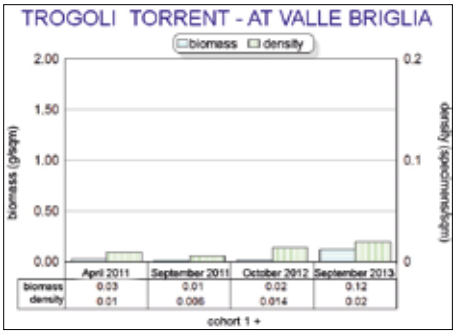


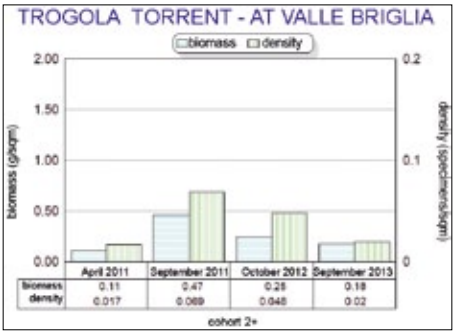
Tab. 33 – Biomass and density values for *C. gobio*, estimated during quantitative monitoring during the 2011/2013 three year period.

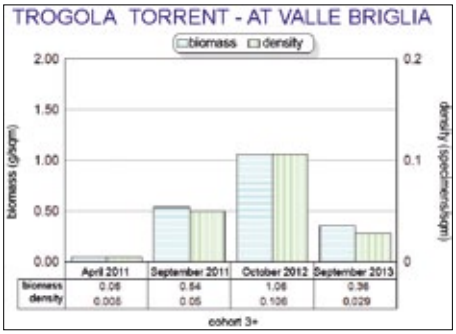
The diagram shows how both parameters showed a positive trend up until October 2012, with a biomass of 1.76 g/m² and a density of 0.214 ind/m²; the semi-quantitative abundance indicator attributed the European bullhead with a value that varied from 5 (dominant) during the 2011 censuses, to 4 (abundant) in September 2013. In any case, the population appears structured.

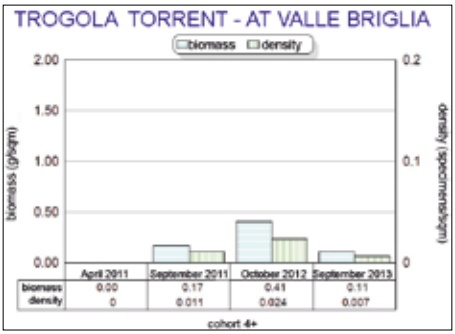
The age classes present range from 0+ to 5+; the diagrams below show the estimated biomass and density values for the European bullhead over the years, divided by cohort.

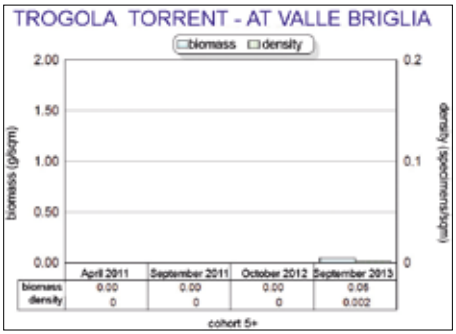










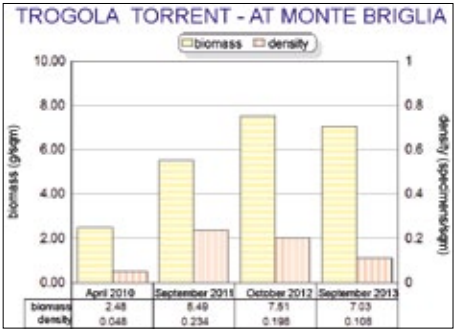


Tab. 34a, b, c, d, e, f – Biomass and density by class in *C. gobio* in TR_02.

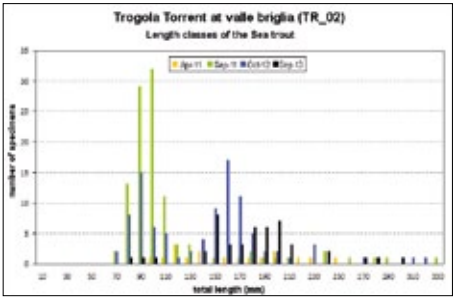
The following tables summarise the data on estimated density and biomass for the brown trout during the monitoring activities conducted in the 2011-2013 three year period.

Scientific name	Date	B (g/m²)	D (specimens/m²)	A.I.	Structure
Salmo (trutta) trutta	21/04/2010	2.48	0.048	3	4
	15/09/2011	5.49	0.234	5	1
	02/10/2012	7.51	0.198	5	1
	12/09/2013	7.03	0.108	4	1

Tab. 35 – Biomass and density in S. (trutta) trutta in TR_02 in the 2011-2013 period.



Tab. 36 – Biomass and density values in S. (trutta) trutta estimated during quantitative monitoring conducted in the 2011-2013 three year period.



Tab. 37 – Distribution in length classes of S. (trutta) trutta in the 2011-2013 period.

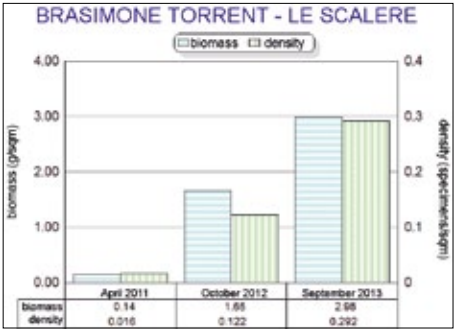
Here too, there was a clear fall in the number of competitors.

In the Bolognese area of the Brasimone stream, the verifications and monitoring were conducted mainly during the phase of the *in-situ* experimentation. A short *excursus* of the results is provided below.

Brasimone torrent - Station BR_02

Scientific name	Date	B (g/m²)	D (specimens/m²)	A.I.	Structure
Cottus gobio	06/04/2011	0.14	0.016	2	4
	02/10/2012	1.65	0.122	4	1
	12/09/2013	2.98	0.292	5	1

Tab. 38 – Biomass and density in *C. gobio* in BR_02 in the 2011-2013 period.

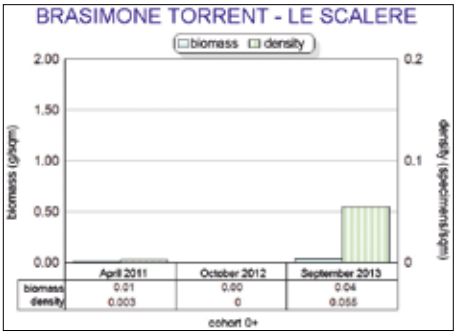


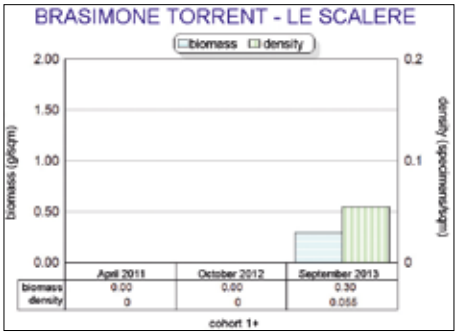
Tab. 39 – Biomass and density values for *C. gobio* estimated during quantitative monitoring conducted in the 2011-2013 three year period.

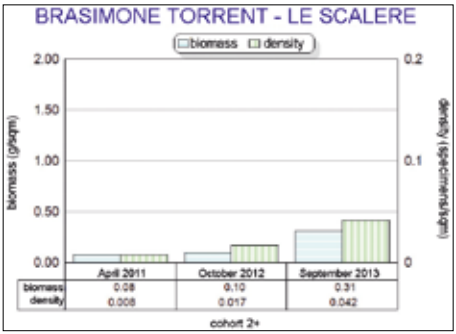
The figure above shows how the population trend for *C. gobio* grew sharply, with biomass and density, updated to September 2013, of 2.98 g/m² and 0.292 ind/m² respectively. According to the semi-quantitative abundance indicator, the species was classed as dominant, with a structured population-

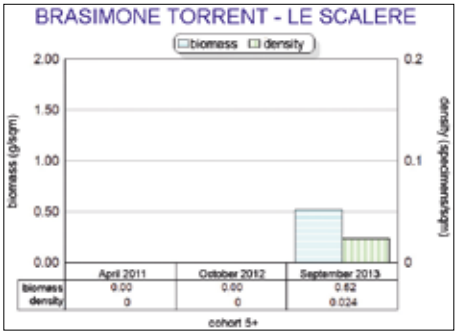
The age classes present range from 0+ to 5+; the diagrams below show the estimated biomass and density values for the European bullhead over the years, divided by cohort.

It is very interesting to note how in this case, the flooding in March 2013 did not have the negative impact observed in the Apennine territory in the Province of Prato, given that it did not directly affect this hydro-graphical basin.









Tab. 40a, b, c, d, e, f – Biomass and density by class of *C. gobio* in BR_02.

In the site in question, the censuses conducted of this species were qualitative; the data are therefore expressed in terms of the abundance indicator (A.I.) and population structure.

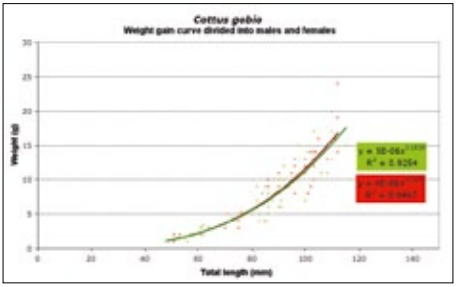
Scientific name	Date	B (g/m²)	D (specimens/m²)	A.I.	Structure
Salmo (trutta) trutta	06/04/2011	-	-	5	1
	02/10/2012	-	-	4	3
	12/09/2013	-	-	4	1

Tab. 41 – A.I. and population structure for S. (trutta) trutta in BR_02 in the 2011-2013 period.

The brown trout is a species considered to be abundant in the area of the Brasimone in question, despite being classed as dominant in April 2011; the population appears to be structured.

The general picture emerging from the monitoring conducted by the scientific consultants of the Lakes Suviana and Brasimone Natural Park, in the six sampling stations considered, is of a general improvement in the populations observed which, given the gradual fall in water flow recorded in recent years, should be considered in relation to the application of trout repopulation policies adapted to the ecological characteristics of the areas examined. The populations covered by the census appear well-structured and abundant, in particular on the Limentra di Treppio, where the species had a good overall level of conservation, in an area characterised by intact substrates, a limited slope, a good width of riverbed and strong water flow. The latest monitoring activities conducted in 2013 showed a significant increase in the abundance and density of the species both in the Limentra di Treppio and the Brasimone, with a strong presence of specimens in the earlier age classes (0+ e 1+). The surveys conducted as part of this project have allowed us to collect numerous biometric data on *C. gobio* in the water courses covered. In total, we collected data on more than a thousand specimens.
The following diagram shows the weighted growth curves for the population of *C. gobio* divided by gender. In order to construct the curves, we used the data from the monitoring activities conducted during the reproductive period, when the distinctive morphological features of each gender are most clear. For the females of the species (in green) the relationship is expressed with the following linear equation, where P is the weight in grams and L is the total length in mm: log (P) = -5.2746 +3.1639 Log (L), with a correlation coefficient of 0.9254, while for the males (in red) we have log (P) = -5.3773 +3.2218 Log (L), with a slightly higher correlation coefficient of 0.9467.

The weighted growth curves for the two genders match entirely, with a slightly higher level of fitness in the females.



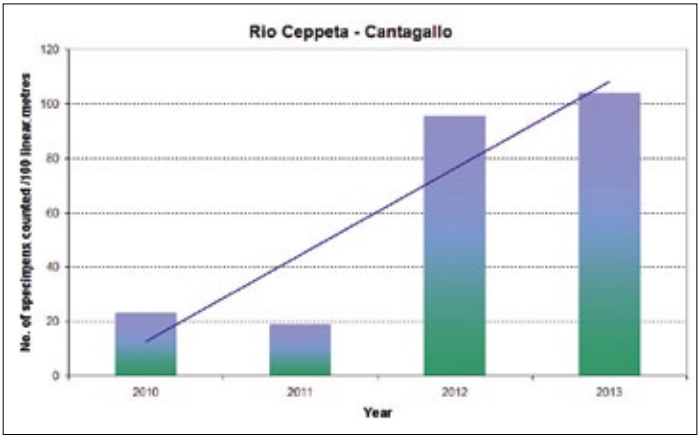
Tab. 42 – Weighted growth curves for *C. gobio* divided by gender.

Monitoring the White-clawed crayfish

The monitoring of *A. pallipes* started in 2010 with preliminary studies and sampling of reproducers both for genetic profiling and to build up the *ex-situ* reproduction pool.
During February 2011, we therefore laid the artificial substrates and manually adapt the sites (using material found in loco) for the creation of reproductive sites and *in-situ* refuge areas, suitable for reproduction and weaning. These sites were inspected and maintained in function throughout 2012. For further details, please refer to the relative paragraph.
The results of the monitoring activities for *A. pallipes* as part of the experimentation are summarised in the tables below.

Scientific name	Period of investigation	Specimens captured in 100/ML
Austropotamobius pallipes	2010	23
	2011	19
	2012	96
	2013	104

Tab. 43 – Results of the monitoring activities for *A. pallipes*.



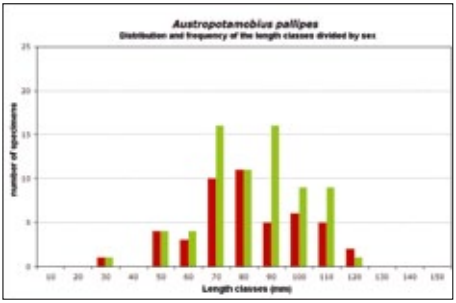
Tab. 44 – Specimens of *A. pallipes* recorded during the monitoring activities between 2010 and 2013.

In this regard, we note that the cumulative effect of the numerous variables involved, such as the exceptional weather events which took place during the experimentation, or tampering by unknown persons, has made it difficult to record and interpret the results.
In order to align the results from the various years and make it possible to perform a critical analysis of them, we processed them by calculating the number of specimens in 100 linear metres for all categories. In the case of more than one census performed in the same year, we took the highest number recorded. Where different techniques were used in the same area on the same day, we totalled the results from the different survey techniques (fish traps + manual surveys).
Regardless of the resulting values, it is important to note that we saw a net improvement in the size of the population in 2012 and 2013, after launching the experimentation in 2011, demonstrating the project's success.

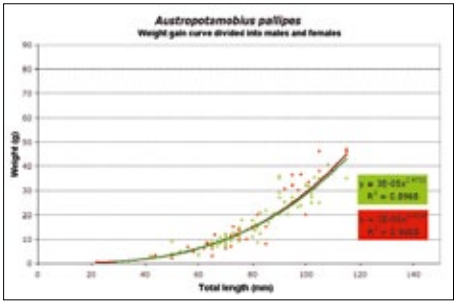
The activities conducted on the Rio Ceppeta, alongside the *ex-situ* work, also allowed us to identify some good practices for breeding in captivity, starting with wild, genetically selected breeding stock, and for putting the know-how acquired in reproduction within the species into practice.
In addition to these operations, we would also like to consider the battle with competitors, in this case the brown trout, the introduction of which was stopped within the areas of the experimentation and considerably reduced in the surrounding areas, limiting the new introductions to juvenile material. This factor may also have affected the successful outcome of the experimentation.

The situation on the Bolognese area of the river, where the White-clawed crayfish did not show any noticeable improvement in its conservation status within the area monitored, was rather different. The presence of the species, verified during monitoring, remained rare and limited to a few stations, demonstrating highly fragmented distribution. Evidence of crayfish plague (Aphanomyces astaci) found at the sampling station located on the Rio del Bago (monitoring conducted during 2011) could explain the lack of crayfish during the subsequent sampling activities. The situation deserves particular attention, given that the plaque represents one of the main causes of the decline in *A. pallipes* in the whole area of the species' distribution.
The tables below set out the frequency distribution, divided by length class at intervals of 10 mm, of *A. pallipes* specimens, divided by gender, captured during the sampling performed during the project. As shown in Tab. 045, the female specimens were more common in nearly all the length classes, and in particular above 90 mm. The length classes with the lowest presence of males were 70 and 80 mm.

The diagram below shows the weighted growth curves for the *A. pallipes* population, divided by gender: the females in green and the males in red. For the females of the species the relationship is expressed with the following linear equation, where P is the weight in grams and L is the total length in mm: log (P) = -4.4897 +2.9721 Log (L), with a correlation coefficient of 0.8968, while for the males we have log (P) = -4.4639 +3.0531 Log (L), with a slightly higher correlation coefficient of 0.9008. It is clear how the curves match each other perfectly.



Tab. 45 – Frequency distribution by length classes for *A. pallipes.*



Tab. 46 – Weighted growth curves for *A. pallipes* divided by gender.

Checking the functionality of the fish ladders

In order to evaluate the functionality of the fish ladders created on the Rio Ceppeta, on the evening of 01 October 2012, after first cleaning the ladder, we blocked the exit channel of the last basin (the 10th) flowing downstream using 1cm netting, and introduced a total of 10 specimens of *C. gobio*, 6 of *Salmo (trutta) trutta* and 3 of *A. pallipes*, all large enough not to be able to pass through the net and follow the flow downstream.
The following morning, we checked the fish ladder basins by hand and using a fish stunner, in order to see whether the specimens introduced had managed to climb the ladder; for the sake of clarity, we note that the fish ladder on the Rio Ceppeta is formed of 10 subsequent basins.
Of the 10 specimens of *C. gobio* introduced, 3 were found in the 10th basin (where they were released), 1 in the 8th, 1 in the 4th and 1 in the 2nd basin, close to the exit of the fish ladder. Regarding *S. (trutta) trutta,* once again 2 specimens were found in the 2nd basin. We weren't able to find the *A. pallipes* specimens.
Based on the results obtained, we believe that the fish ladder is fully functional for the purpose, as most of the specimens released succeeded in getting past the obstacle created by the dam in only a few hours. Moreover, the results were very positive and encouraging given the water flow measured when performing the test, which was particularly high and therefore not optimal for the functionality of the fish ladder.
A similar evaluation of the functionality of the fish ladder on the Trogola-Alto Bisenzio was performed on 2 October 2012. After first cleaning the work, the exit of the last basin downstream was closed up using 1 cm netting and we then introduced a total of 20 specimens of *C.gobio* to the last basin (the 10th), all large enough to prevent them from flowing through the netting downstream.
The *C.gobio* specimens used were among those surveyed at the TR_02 station: in order to make them easier to recognise when climbing the ladder, we marked the specimens with *Alcian Blue* colouring using a *Panjet* inoculator.

4 hours after releasing them, we manually checked basins 11 to 7 on the fish ladder. Of the 20 marked specimens of *C. gobio* introduced, 6 were found in the 11th basin (where they were released), 1 in the 10th, 1 in the 9th and 1 in the 8th basin.

Fig. 88 – Marking the *C. gobio* specimens.

Fig. 89 – *C. gobio* specimen marked using *Alcian Blue* colouring.

Using the results obtained, we believe that the fish ladder is functioning well, confirmed by the fact that the *C. gobio* specimens introduced at the bottom of the ladder had already started successfully climbing the ladder after only a few hours. Moreover, the results of this experiment should be considered in light of the higher-than-project-average water flow at the time, which certainly did not help the ladder to function correctly. We therefore believe that the ladders are fit for the purpose for which they were constructed.

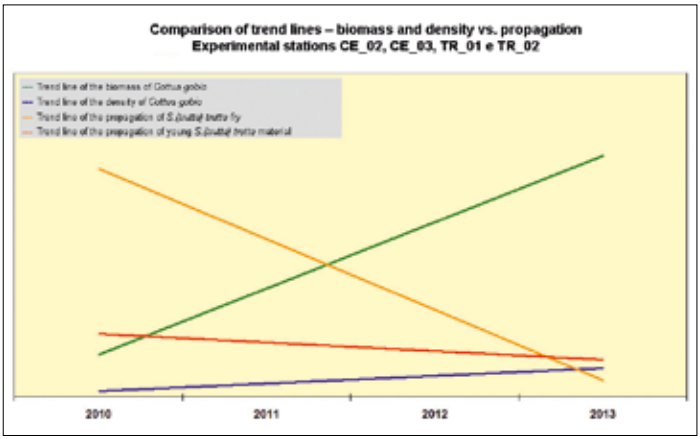
Effectiveness of active conservation measures for the European bullhead and White-clawed crayfish

From the findings set out in the paragraphs above, we drew the following conclusions:

- The experimentation as a whole, as shown by the results obtained, despite the short period during which it was conducted, was very successful;
- The biological monitoring showed a positive synergy effect of experimenting with several actions at once (*in-situ*, *ex-situ*, reconstructing water continuity) affecting the population size of the two target species, *C. gobio* and *A. pallipes*;
- The beneficial effect was also seen on the population structure, involving the entire range of cohorts;
- Given the limitations imposed by the Action Plan, we saw a clear fall in the number of predators present, specifically *Salmo (trutta) trout.*

This evaluation is well represented in the following diagram which, considering the various areas of experimentation as a whole, gives an overview of the species data. It is clear how the fall in the number of predators corresponds to a growth in the indigenous population of *C. gobio*.

Tab. 47 – Biomass and density trend for *C. gobio* and brown trout seeding in the stations covered by the project.



We should, however, clarify that without the combined effect of all the other actions taken, the phenomenon would not have been so clear.
Other aspects that were highlighted by the biological monitoring:
Catastrophic events of an intensity of around 80/100 mm had a clear short-term impact on *C. gobio*, while *A. pallipes* did not appear to be equally affected;
The verification of the functionality of the fish ladders gave good results using the technique of introducing a well-defined number of specimens directly at the bottom of the ladder and closing the downstream exit off, while the results using the *Panjet* marking were almost insignificant upstream of the fish ladder (a single specimen was found on the Rio Ceppeta in 2012).
In this respect, we note that the marked specimens were not found downstream either, in the area where they were released after the tattoo. This shows that the use of these techniques requires a large number of specimens to be used, which is difficult to guarantee in the wild and especially in populations of this species, where it is possible to mark, without harming the fish being monitored, only those over the size of 10 cm (upper margin of the 3+ cohort).

Biological monitoring of herpetofauna

The core of the work consisted of searching for the target species *Triturus carnifex* within the wetland areas for requalification.
Although the species has historically been present in the plain between Prato and Florence, none were found after a careful analysis of the detailed data provided by the Province for their territory, and this absence was confirmed by the fauna surveys conducted as part of this project.
Thanks to an action based on the so-called *Expert-based* method, involving meetings with local experts (Andrea Vannini - Biologist, Giacomo Bruni - Final year Biology student, and Fabrizio Gallotta - Fauna expert), we extended the survey field to include other locations surrounding the SPA. This lead to the finding of vital populations of *Triturus carnifex* in the new sites (named site 04 – Fossi di Castelnuovo and site 05 – Stagni di S. Ippolito).
The adult specimens of *Triturus carnifex* and *Lissotriton vulgaris* found were identified by their pattern of dark patches on the belly, and recorded in a special database: the belly pattern represents a genuine “fingerprint” for the species, and is unique82 in each individual.

During the monitoring, the pools that were artificially constructed as part of the project at the 03 Lake Ombrone site were the only habitats suitable for introducing the crested newt, due to the absence of their alien invasive competitor *Procambarus clarkii*. In order to protect this species, the artificial pool in site 03 was therefore used as a source area for developing *Triturus carnifex* populations, allowing them to radiate outwards from this site and recolonize the surrounding agricultural ecomosaic.
Once vital populations of the target species had been located, although in areas that were not entirely suitable and in the presence of important threats, we proceeded to extract and transfer larvae and adult specimens mainly of *Triturus carnifex* (Annex II and IV of the "Habitat" Directive 92/43/EEC, Annex II Bern Convention, Annex A L.R.T. 56/00) and *Lissotriton vulgaris* (Annex III Bern Convention, Annex A L.R.T. 56/00), after obtaining specific authorisation from the Ministry of the Environment and Protection of Land and Sea, on the opinion of ISPRA and the SHI.
The ISPRA authorisation highlighted the need to transfer not only adult specimens, but also larvae, so that they could undergo metamorphosis at the destination site.
The paragraphs below set out the results of the herpetological surveys of the sites monitored during the 2010-2013 period.

Site 01 – Lake Pantanelle

Site 01 Lake Pantanelle										
	2010		2011		2012		2013		Totale	
Species	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.
Hyla intermedia	0	-	0	-	0	-	4	16,0	4	3,7
Lithobates catesbeianus	0	-	6	23,1	0	-	1	4,0	7	5,9
Pelophylax bergeri / P. kl. *hispanicus*	20	100,0	20	76,9	47	100,0	20	80,0	107	90,7
Totale	20	100	26	100	47	100	25	100	118	100

Tab. 48 – Numeric results (Number of Specimens) and frequency (F% nd.) in the herpetological surveys (Site 01).

The actions performed at this site, in line with the LIFE project, led to the construction of 2 hatchery-pools for amphibians, exclusively rainwater fed, which are highly likely to have positively influenced biodiversity in general and the conservation of this important group of fauna in particular (especially the *Pelophylax bergeri/P. kl. hispanicus* species). This is demonstrated both by the increasing numbers over time, and by the fact that the local population of Italian pool frogs (*Pelophylax bergeri/P. kl. hispanicus*) was found to be well structured and numerous, despite the presence of invasive alien species (in particular *Procambarus clarkii* and *Lithobates catesbeianus*). These hatchery pools have not been used in the past to host *Triturus carnifex* precisely because of the presence of numerous specimens of *P. clarkii*, found in these sites, despite the technical design features included to prevent them from entering: the access methods used by the invasive alien species in question are still unknown.
The *Pelophylax bergeri/P. kl. hispanicus* specimens monitored showed a clear ecological preference for the hatchery pools and for the southern part of the lake, avoiding the NE area and the wetlands to the north of the lake, where the population fell decisively following the invasion of these sub-areas by large populations of *P. clarkii*.

Site 02 – Lake Bogaia

Site 02 Lake Bogaia										
	2010		2011		2012		2013		Totale	
Species	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.
Hyla intermedia	0	-	0	-	0	-	4	36,4	4	16,0
Pelophylax bergeri / P. kl. *hispanicus*	10	100,0	0	-	4	100,0	7	63,6	21	84,0
Totale	10	100	0	0	4	100	11	100	25	100

Tab. 49 – Numeric results (Number of Specimens) and frequency (F% specimens) in the herpetological surveys (Site 02).

This wetland area, in contrast to Lake Ombrone and Lake Pantanelle, is characterised by the absence of any artificial devices to regulate water levels, as it is fed by the overflow from the phreatic zone, linked to seasonal factors (quantity and duration of rainfall). In fact, during the period monitored, the water level changed frequently, with an obvious effect on the results: for example, after a prolonged summer drought in 2012, the wetlands were divided into three, unconnected pools.
The works to expand and remodel the morphology of the lake and its banks did effectively improve the ecological conditions for the amphibians, as demonstrated by the increase in the number of species found. Nonetheless, we expect conditions to improve further, given that the effects of the interventions should begin to become more apparent after the vegetation takes hold on the banks (phragmites, bushes), which will require at least one vegetative season after planting. The environment was evaluated as of medium suitability for the target species *T. carnifex*, although the species was not found during the monitoring activities performed.

Site 03 – Lake Ombrone

Site 03 Lake Ombrone										
	2010		2011		2012		2013		Totale	
Species	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.	N°count. Spec.	F% Spec.
Hyla intermedia	0	-	0	0,0	0	-	4	13,8	4	3,3
Pelophylax bergeri / P. kl. *hispanicus*	10	100,0	40	100,0	42	100,0	25	86,2	117	96,7
Totale	10	100	40	100	42	100	29	100	121	100

Tab. 50 – Numeric results (Number of Specimens) and frequency (F% specimens) in the herpetological surveys (Site 03).

In this site, the dominant amphibian species over the course of the years has remained *Pelophylax bergeri/ Pelophylax kl. hispanicus*. The community of amphibians appears to be considerably smaller than the potential in the area, where the target species *T. carnifex* could also find room. In fact, there is a network of canals and ditches along the borders of the agricultural areas to the Northwest of the lake, which has considerable potential for the species in question, although the species has never been found there during several years of monitoring activities.
The use of morphological markers (patterns of belly patches) has allowed us to record the variation in distribution of specimens over time and space. This has allowed us to observe how in months with higher rainfall (March-April) the specimens monitored prefer to remain in the ditches around the borders of Lake Ombrone, rather than in the lake itself, presumably in order to avoid predation by birds, or because the habitat is simply

more suitable for survival. The Frog population partly withdraws to the lake when the water in the ditches starts to dry out and shortly afterwards moves to its summer refuge sites.
Both in 2012 and 2013, *Pelophylax* showed a tendency to colonise the hatchery pools specially created as part of the "Water SCIs" LIFE project, demonstrating the validity and efficacy of the work done. The population trend for *Pelophylax* certainly influence the larval development of *T. carnifex*, transferred to this site in the spring of 2013: the project aims to achieve a balance between the populations of the two species and establish a source area for reconstructing vital populations of *T. carnifex*, protected from the threats in the sites where they are currently found. The ecological factors that are important for the establishment of the *T. carnifex* larvae include:

- The presence of vegetation coverage and in particular aquatic plants (the reason for the artificial introduction of specimens of *Ceratophyllum demersum*, *Miriophyllum spp.*, *Chara sp.* and *Potamogeton sp.*);
- Predatory activity by *Dytiscidae* beetles and *Odonata* larvae;
- Constant "*mobbing*" of the "new arrivals" by *Pelophylax bergeri/P. kl. Hispanicus* larvae present in the hatchery pools.

Site 04 – Fossi di Castelnuovo

Site 04 Fossi di Castelnuovo						
	2012		2013		Totale	
Species	**N°count. Spec.**	**F% Spec.**	**N°count. Spec.**	**F% Spec.**	**N°count. Spec.**	**F% Spec.**
Lissotriton vulgaris	8	72,7	4	66,7	12	70,6
Triturus carnifex	3	27,3	2	33,3	5	29,4
Totale	11	100	6	100	17	100

Tab. 51 – Numeric results (Number of Specimens) and frequency (F% specimens) in the herpetological surveys (Site 04).

The local population of the target species *Triturus carnifex* is well structured with specimens from both genders and numerous larvae. The reproductive capacity of the target species leads us to think that site 04 is a suitable site of preference for the target species in the Prato area.
The *Lissotriton vulgaris* population appears to have a good level of conservation, despite the lower number of larvae found.
After the monitoring, we proceeded, with ministerial authorisation, to capture and transfer larvae and adult specimens, mainly of *T. carnifex* (1 adult and 34 larvae) and *Lissotriton vulgaris* (1 adult and 2 larvae) to the hatchery pools created for the purpose as part of the "Water SCIs" LIFE project for adults and larvae from these species.

Site 05 – S. Ippolito Ponds

Site 05 S. Ippolito Ponds				
	2013		Totale	
Species	**N°count. Spec.**	**F% Spec.**	**N°count. Spec.**	**F% Spec.**
Hyla intermedia	10	30,3	10	30,3
Lissotriton vulgaris	2	6,1	2	6,1
Triturus carnifex	1	3,0	1	3,0
Pelophylax bergeri / P. kl. *hispanicus*	20	60,6	20	60,6
Totale	33	100	33	100,0

Tab. 52 – Numeric results (Number of Specimens) and frequency (F% specimens) in the herpetological surveys (Site 05).

In comparison to site 04, which was classed as suitable, despite a strong anthropic impact, site 05 was in a good state of conservation: although found in an agricultural context, the area was shown to be an important source area for the *T. carnifex* population in the Prato plains territory.
The local population of the target species *T. carnifex* was in a good state of conservation and well-structured with abundant numbers of adults and larvae, despite the threat of invasive alien species, in particular *Procambarus clarkii*, also found here.
The *Lissotriton vulgaris* population appears to have a good level of conservation, despite finding fewer larvae than for *T.Carnifex*.
Pelophylax bergeri/P. kl. hispanicus and *Hyla intermedia* both had large populations composed of numerous adults and a good number of larvae (higher in *Pelophylax* than in *Hyla*).
Moreover, among the wide range of aquatic vegetation present, the site also includes some species of flora and fauna of regional interest, to the point that if extended, it will meet the criteria for inclusion in the SIC/SPA/SIR "Ponds of the Florentine and Prato plain" list alongside the wetlands located just to the north of Lake Pantanelle.
After finding the species, we proceeded, with ministerial authorisation, to capture and transfer 39 *T.Carnifex* larvae and 1 *L.vulgaris* larva to the hatchery pools created for the purpose as part of the "Water SCIs" LIFE project. In this case, no adult specimens of the Italian crested newt were transferred.

Effectiveness of active conservation measures for herpetofauna
From the results obtained for each site, we saw a considerable improvement in the biodiversity of the herpetofauna in terms of the number of species and the abundance of the populations monitored. The actions taken to date are therefore considered on the whole to have been successful and should be continued.
In particular, the experimental classification system used (marking specimens by photographing the characteristic pattern of dark patches on a yellow-orange background of the belly of *T. carnifex and L. vulgaris*) was particularly suitable for evaluating the effectiveness of the concrete conservation measures concluded. This method facilitates the adaptation of the sites of interest: monitoring the demographic trend and modifications in the topographic distribution of the populations of amphibians present, which are extremely susceptible to variations in environmental parameters and the intensity of disturbances, allowed us to intervene rapidly in the case of any fall in the population, between one herpetological survey and the next.

Biological monitoring of birdlife

As required by international standardised methodology, we used the direct counting method for monitoring birdlife, counting all the specimens present in a certain area, or estimating the numbers in the case of large flocks. The totals are therefore the result of adding the estimates and the absolute and/or partial counts, without any rounding up or other transformations[15,83].
Direct surveys were conducted by observing the species present both from fixed positions (observatories) and on foot along routes inside the area, taking care not to disturb the aquatic birdlife in the pools, in order to avoid making them move to outlying areas.
The surveys were conducted from dawn to midday each day of the census. Among the detectors that have contributed to data collection, the expert ornithologist Alessio Bartolini.
The findings were aggregated according to the following parameters:
- Number of contacts by type (song, call, observation): this division by type of contact was particularly important during the spring reproduction period, as it allowed us to hypothesise nesting for the species identified by their song;

- Total number of contacts divided by species;
- Check list of high priority conservation species found, primarily based on Annex I of Directive 2009/147/EC, and secondly on other classifications, in particular the LRUNI = 2011 Red List of Breeding Birds in Italy, annexed to Regional Law 56/00.

In order to understand the tables below, we used the following classifications for the state of conservation, and the following abbreviations:

1. IUCN Red List of Threatened Species - International Union of Conservation Nature Global conservation status:
   - Extinct, EX
   - Extinct in the Wild, EW
   - Critically Endangered, CR
   - Endangered, EN
   - Vulnerable, VU
   - Near Threatened, NT
   - Least Concern, LC
   - Data Deficient, DD
   - Not Evaluated, NE

2. SPEC Classification = Species of European Concern
   - SPEC = Species of European Concern
   - SPEC 1: species of global conservation concern (i.e. their conservation status is classified as under threat on a global level).
   - SPEC 2: species with an unfavourable European conservation status, and with more than half of the global breeding or wintering population concentrated in Europe
   - SPEC 3: species with an unfavourable European conservation status, but with less than half of the global breeding or wintering population within Europe
   - Non SPEC: species with a favourable conservation status
   - Non SPEC^{E}: species with a favourable European conservation status, but with more than half of the global breeding or wintering population concentrated in Europe

3. ETS Classification = European Threatened Species (BirdLife International, 2004). Conservation status:
   - CR Critically Endangered
   - EN Endangered
   - VU Vulnerable
   - D Declining
   - R Rare
   - H Depleted
   - L Localized
   - DD Data Deficient
   - S Secure
   - NE Not Evaluated
   - () provisional status

4. Directive 2009/147/EC List of annex 1 = inclusion in the shortlist of priority conservation species present in Annex I of EC Directive 2009/147/EC

5. LRUNI Classification = 2011 Red List of Breeding Birds in Italy
   - NA = Not applicable
   - EX = Extinct
   - EW = Extinct in the Wild
   - RE = Extinct within the Region
   - CR = Critically endangered
   - EN = Endangered
   - VU = Vulnerable
   - NT = Near threatened
   - LC = Least Concern
   - DD = Data Deficient
   - NE = Not Evaluated

6. Regional Law 56/00 = inclusion in Annex A2 of Tuscany Regional Law n. 56/2000 “Regulations for conservation and protection of natural and semi-natural habitats, wild flora and fauna

The conservation status of the individual target species, recorded during 2013 after the environmental requalification of the wetland areas was completed, was evaluated taking account of the size of the populations found in the previous direct surveys (*ex-ante*) conducted as part of the "Water SCIs" LIFE project.
The scale used to evaluate the conservation status can be summarised in the following categories: red :-(, amber :-|, green :-)
Alongside the performance of the conservation status, we also summarise the main threats and species-specific conservation measures to be taken in order to maintain the current conservation status.

Checklist for species of major conservationist interest found at Lake Pantanelle – Site 01 – period april-december 2013									
N.	**Euring**	**English name**	**Scientific name**	**IUCN**	**SPEC**	**ETS**	**Dir. 2009/147 EC List of annex 1**	**RLNBI**	**Regional Law 56/00 All. A2**
1	00980	Little Bittern	*Ixobrychus minutus*	NT	SPEC 3	(H)	-	VU	SI
2	01040	Black-crowned Night Heron	*Nycticorax nycticorax*	LC	SPEC 3	H	-	VU	SI
3	01080	Squacco Heron	*Ardeola ralloides*	LC	SPEC 3	(D)	-	LC	SI
4	01190	Little Egret	*Egretta garzetta*	LC	Non-Spec	S	-	LC	SI
5	01210	Great Egret	*Casmerodius albus*	LC	Non-Spec	S	-	NT	SI
6	01240	Purple Heron	*Ardea purpurea*	LC	SPEC 3	(D)	-	LC	SI
7	01340	White Stork	*Ciconia ciconia*	LC	SPEC 2	H	-	LC	-
8	02020	Ferruginous Duck	*Aythya nyroca*	NT	SPEC 1	(VU)	SI	EN	SI
9	02600	Western Marsh Harrier	*Circus aeruginosus*	LC	Non SPEC	S	-	VU	SI
10	04550	Black-winged Stilt	*Himantopus himantopus*	LC	Non SPEC	S	-	LC	SI
11	05170	Ruff	*Philomachus pugnax*	LC	SPEC 2	(D)	-	-	-
12	05540	Wood Sandpiper	*Tringa glareola*	LC	SPEC 3	H	-	-	-
13	05780	Little Gull	*Larus minutus*	LC	SPEC 3	(H)	-	-	-
14	08310	Common Kingfisher	*Alcedo atthis*	LC	SPEC 3	H	-	LC	SI

Tab. 53 – Checklist of high priority conservation species in site 01 (Lake Pantanelle).

Checklist for species of major conservationist interest found at Lake Bogaia – Site 02 - period april-december 2013									
N.	**Euring**	**English name**	**Scientific name**	**IUCN**	**SPEC**	**ETS**	**Birds Directive 2009/147 EC List of annex 1**	**RLNBI**	**Regional Law 56/00 R. T. All. A2**
1	01040	Black-crowned Night Heron	*Nycticorax nycticorax*	LC	SPEC 3	H	-	VU	SI
2	01190	Little Egret	*Egretta garzetta*	LC	Non-Spec	S	-	LC	SI
3	15150	Red-backed Shrike	*Lanius collurio*	LC	SPEC-3	(H)	-	VU	SI

Tab. 54 – Checklist of high priority conservation species in site 02 (Lake Bogaia) .

Checklist for species of major conservationist interest found at Lake Ombrone – Site 03 – period april-december 2013									
N.	**Euring**	**English name**	**Scientific name**	**IUCN**	**SPEC**	**ETS**	**Birds Directive 2009/147 EC List of annex 1**	**RLNBI**	**Regional Law 56/00 R. T. All. A2**
1	01040	Black-crowned Night Heron	*Nycticorax nycticorax*	LC	SPEC 3	H	-	VU	SI
2	01190	Little Egret	*Egretta garzetta*	LC	Non-Spec	S	-	LC	SI
3	01210	Great Egret	*Casmerodius albus*	LC	Non-Spec	S	-	NT	SI
4	01340	White Stork	*Ciconia ciconia*	LC	SPEC 2	H	-	LC	-
5	01440	Eurasian Spoonbill	*Platalea leucorodia*	LC	SPEC 2	R	-	VU	SI
6	04550	Black-winged Stilt	*Himantopus himantopus*	LC	Non SPEC	S	-	LC	SI
7	05540	Wood Sandpiper	*Tringa glareola*	LC	SPEC 3	H	-	-	-
8	08310	Common Kingfisher	*Alcedo atthis*	LC	SPEC 3	H	-	LC	SI

Tab. 55 – Checklist of high priority conservation species in site 03 (Lake Ombrone).

Checklist for species of major conservationist interest – period april-december 2013									
N.	**Euring**	**English name**	**Scientific name**	**IUCN**	**SPEC**	**ETS**	**Dir. 2009/147 EC List of annex 1**	**RLNBI**	**Regional Law 56/00 All. A2**
1	00980	Little Bittern	*Ixobrychus minutus*	NT	SPEC 3	(H)	-	VU	SI
2	01040	Black-crowned Night Heron	*Nycticorax nycticorax*	LC	SPEC 3	H	-	VU	SI
3	01080	Squacco Heron	*Ardeola ralloides*	LC	SPEC 3	(D)	-	LC	SI
4	01190	Little Egret	*Egretta garzetta*	LC	Non-Spec	S	-	LC	SI
5	01210	Great Egret	*Casmerodius albus*	LC	Non-Spec	S	-	NT	SI
6	01240	Purple Heron	*Ardea purpurea*	LC	SPEC 3	(D)	-	LC	SI
7	01340	White Stork	*Ciconia ciconia*	LC	SPEC 2	H	-	LC	-
8	01440	Eurasian Spoonbill	*Platalea leucorodia*	LC	SPEC 2	R	-	VU	SI
9	02020	Ferruginous Duck	*Aythya nyroca*	NT	SPEC 1	(VU)	SI	EN	SI
10	02600	Western Marsh Harrier	*Circus aeruginosus*	LC	Non SPEC	S	-	VU	SI
11	04550	Black-winged Stilt	*Himantopus himantopus*	LC	Non SPEC	S	-	LC	SI
12	05170	Ruff	*Philomachus pugnax*	LC	SPEC 2	(D)	-	-	-
13	05540	Wood Sandpiper	*Tringa glareola*	LC	SPEC 3	H	-	-	-
14	05780	Little Gull	*Larus minutus*	LC	SPEC 3	(H)	-	-	-
15	08310	Common Kingfisher	*Alcedo atthis*	LC	SPEC 3	H	-	LC	SI
16	15150	Red-backed Shrike	*Lanius collurio*	LC	SPEC-3	(H)	-	VU	SI

Tab. 56 – Complete checklist of target species (Annex I of Directive 2009/147/EC) present in all the wetland areas monitored.

Ferruginous Duck (*Aythya nyroca*): without doubt, the species of the highest conservation interest in the birdlife community, given its priority status as a species of community interest (Directive 2009/147/EC List of annex 1), also classified by BirdLife International as SPEC 1 (conservation status is classified as under threat on a global level, and with more than half of the global breeding or wintering population concentrated in Europe). This is Europe's rarest duck, or more correctly, the rarest duck in the entire paleo-arctic region.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	☹	😐	6
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	☹	☹	-

Tab. 57 – Conservation status of the Ferruginous Duck.

The target species can be found from August to October, only at site 01 Lake Pantanelle (amber conservation status) with 6 observations. This target species should be evaluated over time in order to get a more complete overview of its phenology in the area. In general, the suitability of the sites improved, although the conservation status was only clearly positive in site 01. The species was not found at all in site 02 and site 03 (red conservation status).
Threats: the Ferruginous Duck does not tolerate disturbance by man and pollution, which is always present in delicate ecosystems with stagnant water, such as wetlands.
Priority measures for the species: this target species prefers medium deep water rich in underwater vegetation, with canefields, willows and alders, which we should therefore continue to encourage.

Black-crowned Night Heron (*Nycticorax nycticorax*): This species was found in all three sites monitored (20 obs. Site 01 Pantanelle, 8 obs. site 02 Bogaia, 1 obs. site 03 Ombrone), in particular in site 02 Lake Bogaia where some specimens were observed remaining inactive during the daytime hours, resting in trees at the edges of the wetlands. The species' preferred habitats include flooded willow woodlands.
The species' preferred site in terms of habitat was definitely site 01 Lake Pantanelle, where it was found in abundance, including the presence of juvenile specimens (approx. 8 reported) (green conservation status). Site 02 appeared to be suitable for the target species, given the presence of dormitories (green conservation status), while site 03 was not particularly suited to the species in ecological terms (amber conservation status).

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	☺	☺	20
02	Lake Bogaia	☹	😐	☺	8
03	Lake Ombrone	☹	☹	😐	1

Tab. 58 – Black-crowned Night Heron conservation status.

Overall, the ecological situation in all three sites improved since 2010, thanks to the work done to increase their suitability, such as expanding Lake Bogaia, creating a main island and remodelling the banks and bed of the lake in order to provide a diverse range of depths and therefore meet the needs of a greater number of species, including those in the Ardeidae family.
Threats: see Little Bittern (*Ixobrychus minutus*).
Priority measures for the species: protect reproduction sites (heronries), agricultural good practices. For nesting, like all heron species, it is important to encourage the spread of hygrophilous tree species such as *Populus spp.* and *Salix spp.*

Little Egret (*Egretta garzetta*): This target species was found in all three sites monitored (307 ind. site 01 Pantanelle, 11 obs. site 02 Bogaia, 19 obs. site 03 Ombrone). The Little Egret prefers shallow water with a variety of vegetation, while it prefers flooded willows for nesting.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☺	☺	☺	307
02	Lake Bogaia	☹	☹	☹	11
03	Lake Ombrone	☹	😐	😐	19

Tab. 59 – Little Egret conservation status.

The Little Egret was found most frequently during the course of 2013 in site 01 Lake Pantanelle, where a dormitory is probably located, given the numerous specimens observed feeding (green conservation status). Site 02 could also partly meet the ecological needs of the target species, given the improvements made. Nonetheless, the numbers found were low (red conservation status), probably also due to the fact that the work was concluded in spring-summer 2013 and the expected results have yet to be seen (e.g. development of the phragmites around the lake, growth of vegetation planted, reduction of disturbance/threat factors). In fact, out of 11 observations, 10 were at the nearby detention basin at Ponte a Tigliano. In site 03, the target species was affected by the water level management work underway (amber conservation status). Overall, the ecological situation improved since 2010 in sites 01 and 03, thanks to the work done to make the environment more suitable for the target species.
Threats: see Little Bittern (*Ixobrychus minutus*).
Priority conservation measures for the species: protection of shallow lakes, pools, lagunas and slow-running rivers. The density of the colonies, in this sense, was heavily influenced by the availability of food sources, combined with the availability of suitable sites, usually trees or large bushes, in which to build nests. For nesting, like all heron species, hygrophilous tree species such as *Populus spp.* and *Salix spp.* are important.

Black-winged Stilt (*Himantopus himantopus*): the species was observed at site 01 Lake Pantanelle, where it is breeding (96 obs). In fact, various specimens repeatedly displayed territorial behaviour to protect their nests in this area.
The Black-winged Stilt was also observed (37) at site 03 Lake Ombrone, again displaying territorial behaviour.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☺	☺	☺	96
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	😐	☺	37

Tab. 60 – Black-winged Stilt conservation status.

The target species can be found every year at site 01 Lake Pantanelle (green conservation status) where it is breeding. During 2013 it was found from April to July. It was not found in site 02 (red conservation status), while it was found in site 03 in 2012 and 2013 (green conservation status). In particular, in 2013 it was found in the months of April and August, with territorial behaviour (breeding).
The green conservation status remained constant from 2010 to 2013 in site 01 and improved in site 03 during the same period.
Threats: transformation and destruction of wetland areas with the cutting of vegetation used as refuge, the destruction and modification of riverbeds, chemical and organic pollution of water courses. Important factors to control also include human disturbance of nesting, stopover and wintering sites, as well as pressure from hunting.
Priority conservation measures for the species: protection of breeding sites, protection of colonies from excessive human disturbance, adequate regulation of water levels according to the species' ecological needs. In fact, events such as the sudden drying out or excessive flooding of sites can have a serious effect on a local level[84].

Kingfisher (*Alcedo atthis*): in site 01 Lake Pantanelle there were 2 cases of kingfisher song and 6 direct observations in the period from July-October 2013. In site 03 Ombrone, on the other hand, the species was only observed once.
Site 01 maintained constant results over time and was assessed as suitable for the species (8 obs., green conservation status), site 03 was classified as amber conservation status based on the number of sightings. During 2010-2013, the conservation status for the species improved in sites 01 and 03.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	😐	☹	☺	8
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	☹	😐	1

Tab. 61 – Kingfisher conservation status.

Threats: This target species was under threat from the gradual building development of rivers and streams. Another critical factor was chemical water pollution, which has altered the species' diet, almost completely based on fish.
Priority conservation measures for the species: protection of water courses and wetlands, with particular reference to protecting sandy and earthy banks: this is where the Kingfisher builds its nests, and these are precisely the type of formation that is often swept away during ordinary and extraordinary river maintenance operations.

Red-backed Shrike (*Lanius collurio*): in 2013, this species was found in the areas immediately surrounding site 02 Lake Bogaia (amber conservation status). The northern area of site 01 was also potentially suitable (amber conservation status) although the species was not found here in 2012 or 2013. During 2010-2013, the conservation status for the species was classed as amber in site 01, improved in site 02 and remained red in site 03.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☺	☹	😐	-
02	Lake Bogaia	☹	☹	😐	1
03	Lake Ombrone	☹	☹	☹	-

Tab. 62 – Red-backed Shrike conservation status.

Threats: loss of habitat due to the spread of urbanisation in the plains area. To summarise, a higher level of danger should be attributed to the declining variety of habitats in intensively used and hilly areas.
Priority conservation measures for the species: the Red-backed Shrike needs an environmental mosaic including pastures or farming land alternated with or bordered by bushes or hedges, as found in the areas surrounding the requalified sites covered by the study, which need to be protected.

Squacco heron (*Ardeola ralloides*): observed only in site 01 Lake Pantanelle (9 obs.).

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	☹	😐	9
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	☹	☹	-

Tab. 63 – Squacco Heron conservation status.

The species' preferred site in terms of habitat was definitely site 01 Lake Pantanelle, where a modest number were found despite the good potential suitability of the location (amber conservation status). Despite potentially being an ecologically suitable site, the species was not found at all in site 02 or site 03 (red conservation status). The ecological situation for this species improved since 2010 in all the sites, thanks to the work done to make them more suitably, even though the increase saw a very small rise in terms of numbers of specimens, and then only in site 01 Lake Pantanelle.
Threats: see Little Bittern (*Ixobrychus minutus*).
Priority conservation measures for the species: protection of nesting and foraging sites, continuing monitoring to gain more information on ecological aspects relating to the species. For nesting, it is important to maintain thick bushes or wooded areas.

Great Egret (*Casmerodius albus*): the species was monitored in two sites (36 obs. site 01 Pantanelle and 10 obs. site 03 Ombrone). The species' ideal environment for feeding is shallow waters, while it prefers to nest in large trees.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	☹	☺	36
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	😐	😐	10

Tab. 64 – Great Egret conservation status.

The most suitable location for this target species is definitely site 01 Lake Pantanelle (green conservation status), where it can be found throughout the year. This is mainly thanks to the work done to improve the environment in general and in particular for the target species.
Site 02 was found to be unsuitable for the target species (not found, red conservation status), while the species was classed as amber conservation status in site 03, where it was only found from April to June in 2013. The conservation status is improving in sites 01 and 03.
Threats: see Little Bittern (*Ixobrychus minutus*).
Priority conservation measures for the species: protection of the wetlands, with a preference for more extended marshy ground, where the target species builds its nests in thick, mostly inaccessible, canefields. The nests are usually built directly on the water, or not more than 4-5 metres off the ground. The demographic increase in Austrian and Hungarian populations, thanks to the heronry conservation measures, has had a positive impact on the numbers of this species in Italy[84].
For nesting, like all heron species, it is important to encourage the presence of hygrophilous tree species such as *Populus spp.* and *Salix spp.*

White Stork (*Ciconia ciconia*): the species was observed in June and July 2013 at site 01 Lake Pantanelle (3 ind.) and at site 03 Lake Ombrone (5 obs.). From 2011, the species was found to be consistently breeding in site 03.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	😐	☺	3
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	😐	☺	5

Tab. 65 – White Stork conservation status.

The conservation status of the target species was green in sites 01 and 03 while it was red in site 02. The conservation status improved in sites 01 and 03, and the general ecological suitability for the species improved in all three sites.
Threats: in the past, these involved large-scale marshland recovery operations and change of use for the land in general, the intensification of farming methods and the unsuitability of most modern buildings for nesting, in contrast to the older style houses and belfries. Maintenance work on power lines may also have a high negative impact on reproduction in breeding pairs.
Priority conservation measures for the species: protection of the wetlands, adaptation of high voltage electricity lines, where present.

Western Marsh Harrier (*Circus aeruginosus*): this species was observed outside the official monitoring campaign, in March 2013, at site 01 Lake Pantanelle (both males and females).

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	😐	😐	3
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	☹	☹	-

Tab. 66 – Western Marsh Harrier conservation status.

The target species can be found only at site 01 Lake Pantanelle (amber conservation status). Here too, the presence of the target species should be monitored over time. The species was not found at all in site 02 and site 03 (red conservation status).
During the monitoring period, the conservation status remained amber in site 01 and red in the other two sites.
Threats: destruction of the wetlands and, on a secondary level, direct persecution.
Priority conservation measures for the species: correct protection and management wetland areas and the surrounding agricultural land. By protecting all birds of prey, hunting legislation has contributed to the species' presence[84].

Ruff (*Philomachus pugnax*): this species was observed at site 01 Lake Pantanelle (15 contacts) in April 2013 and in the same period of 2012.
The target species was constantly found throughout 2012 and 2013 at site 01 Lake Pantanelle (green conservation status) and also at site 03 Lake Ombrone in 2012 (amber conservation status). The conservation status for the target species was classified as red in site 02, where it was not found at all during the monitoring.
During 2010-2013, the conservation status for the species improved in sites 01 and 03.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	☺	☺	15
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	😐	😐	-

Tab. 67 – Ruff conservation status.

Threats: see Black-winged Stilt (*Himantopus himantopus*). However, the risk of hunting is also a factor, given that the species is in the list of those for hunting as per Law n. 157/92. As a result, it would be useful to know the exact numbers killed by hunters in order to draft international counter-measures. In this respect, we should point out that specimens of Ruff (*Philomacus pugnax*) and Tufted duck (*Aythya fuligula*) cannot be killed within the Tuscany Special Protection Areas, under the terms of Regional Decree n. 454/2008, Annex "A" (Conservation measures valid for all Special Protection Areas).
Priority conservation measures for the species: see Black-winged Stilt (*Himantopus himantopus*).

Wood Sandpiper (*Tringa glareola*): observed in 2013 at site 01 Lake Pantanelle (31 contacts) during the April-July period and at site 03 Lake Ombrone (14 obs.) from April to August.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	☹	☺	31
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	😐	☺	14

Tab. 68 – Wood Sandpiper conservation status.

The target species appeared at site 01 Lake Pantanelle in 2013 (green conservation status) while it was not found at all at site 02 (red conservation status); it was found at site 03 during 2012 and 2013 (green conservation status).
During 2010-2013, the conservation status for the species improved in sites 01 and 03.
Threats: the species has suffered from the changes in the environment in its breeding grounds, due to climate change.
Priority conservation measures for the species: protection of both the ecological quality of the main stopover sites and occasional wintering grounds, and more general protection from excessive human disturbance[84].

Purple Heron (*Ardea purpurea*): the target species was only found at site 01 Lake Pantanelle (amber conservation status) in April and August 2013. Moreover, it was found in the northern part of the lake, where wetland bush formations are found. The environmental improvement works certainly contributed to encouraging its presence. The conservation status improved in site 01 and, although the overall ecological situation improved across the board after the work done, site 02 and site 03 are not yet suitable for the target species (species not found, red conservation status).

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	☹	😐	3
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	☹	☹	-

Tab. 69 – Purple Heron conservation status.

Threats: see Little Bittern (*Ixobrychus minutus*).
Priority conservation measures for the species: encouraging the development of tall, thick, hygrophilous vegetation, in particular canefields associated with shallow freshwater, where the species can easily find prey.

Little Bittern (*Ixobrychus minutus*): this target species was found at site 01 Lake Pantanelle (1 song recorded and 5 sightings). The call of the species allows us to hypothesize a small probability of nesting in the area.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	☹	☺	6
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	☹	☹	-

Tab. 70 – Little Bittern conservation status (*Ixobrychus minutus*).

The improvement in the conservation status is thanks to the work done during the LIFE project in site 01 Lake Pantanelle: regulation of water flow entering and leaving the lake, creation of a main island with a surface area of 1,200 m2, with plant species from the *Salix* and *Populus* families, in addition to 3 smaller, elongated islands with an area of approx. 20 m2.
Threats: collision with power cables, transformation and destruction of current and potential breeding grounds, cutting of plants used as refuge, destruction and modification of river beds, chemical and organic pollution of water courses, human disturbance of breeding grounds, illegal tree cutting and pollution from pesticides used in the surrounding agricultural land.
Priority conservation measures for the species: maintenance of hygrophilous vegetation, in particular canefields, along the banks of rivers, lakes and in wetland areas.

Eurasian Spoonbill (*Platalea leucorodia*): the species was signed (9 obs.) in site 03 Lake Ombrone in May 2013. The Spoonbill lives in shallow pools with hygrophilous plantlife.

Code	Water basin	Conservation status 2010	Conservation status 2012	Conservation status 2013	Number of contacts 2013
01	Lake Pantanelle	☹	☹	☹	-
02	Lake Bogaia	☹	☹	☹	-
03	Lake Ombrone	☹	☹	😐	9

Tab. 71 – Eurasian Spoonbill conservation status.

The target species was found only at site 03 Lake Ombrone (amber conservation status). The presence of this target species should be assessed over time in order to understand if these are occasional presences to be attributed to erratic phenomena.
The species was not found at all in site 01 and site 02 (red conservation status). In general, the ecological suitability for the species improved.
Threats: changes in the wetlands, human disturbance, predators and stagnant areas with very low or absent water refreshment.
Priority conservation measures for the species: the species prefers canefields, bushes or trees such as willows or poplars for nesting. Necessary modifications in order to encourage its presence: a high level of protection against disturbance by man and predators. For this reason, the Spoonbill often prefers small islands to the mainland.

Monitoring breeding grounds

In order to classify the breeding probability of each species, we referred to the criteria listed in the table below, used in the "Atlante degli uccelli nidificanti in Italia" (*Atlas of breeding birds in Italy*, currently being written), which sets out three categories: Possible, probable and certain breeders When reading the following paragraphs, remember that s.=song, c.=call, obs.=sighting.

POSSIBLE NESTING	
1	Sighting of the species during its nesting period
2	Presence in its habitat during its nesting period
3	Male in song present during the nesting period, mating calls or drumming heard, parading male seen
PROBABILE NESTING	
4	Pair present in their habitat during their nesting period
5	Territorial behaviour (song, aggressive behaviour with neighbours, etc.) seen in the same territory on two different days, 7 or more days apart
6	Mating behaviour: parade, mating or exchange of food between adults
7	Visit to a probable nesting site. Different from a resting site
8	Cries of alarm or other behaviour that could indicate the presence of a nest or of young birds in nearby
9	Physiological proof: highly vascularized brood patches or eggs present in the oviduct. Observation of a bird in the hand.
10	Transporting material or building a nest; digging a nest-cavity
CERTAIN NESTING	
11	Bird that simulates an injury or that diverts attention, like, ducks, galliformes, shorebirds
12	Recently used empty nest with eggshells of the current season
13	Young birds with down or that have just left the nest and are unable to fly long distances
14	Adult that arrives at, occupies or leaves a nest; behaviour that reveals an occupied nest, the content of which cannot be verified (too high or in a cavity)
15	Adults carrying a faecal sac
16	Adult carrying food for the young birds during its nesting period
17	Egg shells (hatched or recently predated)
18	Nest seen with a brooding adult
19	Nest containing eggs or young birds (seen or heard)

Tab. 72 – Criteria used in the "Guide to breeding birds in Italy" project to classify breeding.

Site 01 – Lake Pantanelle

Of the three sites considered, Lake Pantanelle was the most suitable for breeding among aquatic birds. The other two sites were more affected by the man-made transformation of the territory, and had a lower variety of habitats.

Certain breeding species:

White Heron (*Ardea cinerea*) on one of the larger trees located in the northern area, Coot (*Fulica atra*), Black-winged Stilt (*Himantopus himantopus*).

Probable breeding species:

Little Grebe (*Tachybaptus ruficollis*) (6 c., 8 obs., including juveniles), Garganey (*Anas querquedula*) in the grassy wetland area to the north (2 obs.), Common Moorhen (*Gallinula chloropus*) (2 c. and 97 obs. including some juveniles), Common Cuckoo (*Cuculus canorus*) (5 c. e 2 obs.), Common Nightingale (*Luscinia megarhynchos*)

(3 c., 1 c.), Blackbird (*Turdus merula*) (4 s. e 25 obs.), Cetti's warbler (*Cettia cetti*) (10 s. e 15 ind.), Eurasian Magpie (*Pica pica*) (2 s. e 2 obs.), Sedge Warbler (*Acrocephalus schoenobaenus*) (4 s.), Eurasian Reed Warbler (*Acrocephalus scirpaceus*) (6 s.), Eurasian Blackcap (*Sylvia atricapilla*) (5 s.), Great Reed Warbler (*Acrocephalus arundinaceus*) (9 s. e 2 obs.), European Goldfinch (*Carduelis carduelis*) (2 s.).
Possible breeding species:
Little Bittern (*Ixobrychus minutus*) (1 s., 2 obs.), European Turtle Dove (*Streptopelia turtur*) (1 s.), Eurasian Wryneck (*Jynx torquilla*) (1 s.), Melodious Warbler (*Hippolais polyglotta*) (1 s.), White Wagtail (*Motacilla alba*) (40 obs.), Common Starling (*Sturnus vulgaris*) (10 s. and 6 obs.).

Site 02 – Lake Bogaia
Certain breeding species:
none
Probable breeding species:
European Turtle Dove (*Streptopelia turtur*) (3 s.), Blackbird (*Turdus merula*) (5 s.), Cetti's warbler (*Cettia cetti*) (3 s.), Zitting Cisticola (*Cisticola juncidis*) (3 s.), Eurasian Blackcap (*Sylvia atricapilla*) (2 s.), European Serin (*Serinus serinus*) (7 s.) and European Goldfinch (*Carduelis carduelis*) (3 s.).
Possible breeding species:
Eurasian Bluetit (*Cyanistes caeruleus*) (1 s.).

Site 03 – Lake Ombrone
Certain breeding species:
Black-winged Stilt (*Himantopus himantopus*), White Stork (*Ciconia ciconia*).
Probable breeding species:
Common Nightingale (*Luscinia megarhynchos*) (5 s.), Blackbird (*Turdus merula*) (9 s., 1 obs.), Cetti's warbler (*Cettia cetti*) (8 s.), Great Reed Warbler (*Acrocephalus arundinaceus*) (2 s.), Eurasian Blackcap (*Sylvia atricapilla*) (5 s.), Great Tit (*Parus major*) (2 s.), European Serin (*Serinus serinus*) (6 s.).
Possible breeding species:
Common Moorhen (*Gallinula chloropus*) (1 c., 2 s.).

Effectiveness of active conservation measures for the target bird species

The level of biodiversity found in terms of bird species, measured using the Shannon-Wiener diversity index, showed higher values in site 01 Lake Pantanelle, followed by site 03 Lake Ombrone and site 02 Lake Bogaia. The Evenness index, which measures how close in numbers each bird species were in the three sites, was also higher in site 01 Lake Pantanelle (E=0.73) in comparison to the other two sites, which both had values of round E= 0.64.
This type of analysis shows how, despite the overall artificiality of these locations, all in very developed urban settings, site 01 Lake Pantanelle has a high level of bird species diversity both in terms of number of species and their relative abundance, which is evenly distributed between species in terms of quantity.
Overall, during the fauna surveys conducted in 2011-2013, 94 species were found in the three lakes, in detail: at Lake Pantanelle 72, at Lake Ombrone 66 and Lake Bogaia 40. The order with the greatest variety of species (36) was the Passeriformes which represented 38%, followed by the Charadriiformes (16 sp.), 17%, the Ciconiiformes (11 sp.), 11%, and the Anseriformes (8%).
There were 44 aquatic species that winter in the lakes, divided as follows: Lake Pantanelle 34, Lake Ombrone 33, Lake Bogaia 10. In total, in the areas surveyed, the most common order of wintering birds was the

Charadriiformes with 36% followed by the Ciconiiformes with 25%, then the Anseriformes with 18% and lastly the Gruiformes with 9%. The order of the Falconiformes among the wintering birds was represented by a single species, the Western Marsh Harrier (*Circus aeruginosus*) with a few presences found at Lake Pantanelle. The bird community also included the Ferruginous Duck (*Aythya nyroca*), a rare species considered a high conservation priority.

Breeding species at Lake Pantanelle included: the White heron (*Ardea cinerea*) on one of the larger trees located in the northern area, the Coot (*Fulica atra*), and the Black-winged Stilt (*Himantopus himantopus*) which, along with the White Stork (*Ciconia ciconia*), also breeds in Lake Ombrone.

The results obtained are still partial and subject to improvement, in particular concerning site 02 Lake Bogaia. This is because the environmental requalification works in this site were only completed in summer 2013, and the positive effects of the work done are presumably not yet fully evident. The same consideration applies to the works to construct the detention basin at Ponte a Tigliano, within which, in line with the instructions provided by the Province of Prato during the Environmental Impact Assessment, an additional semi-permanent wetland area was created. It is reasonable to expect that the extension and remodelling of the bed of the water basin, the planting of the banks and the traffic and hunting bans around the lake will start to take effect from the next vegetative and breeding season (from spring-summer 2014 onwards). In the same way, although only a limited amount of changes were made, and in a marginal area, there is considerable room for improvement at site 03 Lake Ombrone, both in terms of its potential (approx. 11 ha of space, in a favourable geographical location and a limited degree of urbanisation in the environment), and due to the way the lake is currently managed, which is entirely inappropriate for the target species: the early emptying of the basin in the springtime and the tilling of the lake bed to prevent spontaneous plant growth. These practices have been specifically banned, under the terms of the site Management Plan, by the Provincial Hunting Regulation approved in 2013.

CONCLUSIONS

Based on the experience gained in the four years of experimentation and monitoring, we have developed some useful indications to guarantee the future maintenance and, if necessary, reconstitution of vital populations of the target species within their natural habitat.

The "Water SCIs" LIFE project has once again demonstrated (as fully documented in the sector literature[85,86,87,88,89,90,91,92,93]) the importance of an adaptive approach *(adaptive management)* for the correct and prudent management of ecosystems.

The following definition of the term "adaptive management" is taken from the WWF online encyclopaedia94: "the management of natural systems and their inter-relations with social systems, based on dynamic, open and flexible approaches, capable of constantly and rapidly modifying pre-established management plans according to changes in the ecological, economic and social conditions".

This way of operating, in contexts such as the semi-natural environment in question, with a high degree of uncertainly and variability (environmental, economic and social), means that once the management goals have been established, actions can be decided on a case-by-case basis according to information gained from monitoring the dynamics of the specific ecosystem over time. It is therefore a way of proceeding that goes beyond simply modifying the ecosystems being managed, with the aim of improving their conservation status (e.g. directing them towards homoeostasis and increasing their resilience), but also aims to gradually accumulate useful information for understanding how they work[95]. Since this is a learning process that benefits from the experience and information collected, it requires constant attention and a search for balance between the need to obtain the best results in the short term, based on the knowledge available, and the effort to acquire knowledge and skills useful for improving the management results over time[96]. Of course, all while taking account of the resources (financial, human and instrumental) actually available in a given period of time.

In semi-natural environments that are strongly influenced by human presence, like those involved in this LIFE "Water SCIs" project (declining from the lowland to the Apennines), this operating method is therefore the only realistic option for maintaining the quantity and quality of services and assets provided for ecosystems in these areas, as mentioned at the beginning of this volume.

GOOD CONSERVATION PRACTICES FOR THE *EUROPEAN BULLHEAD* AND *WHITE-CLAWED CRAYFISH*

In the specific case of the conservation of the two target species *C. gobio* and *A. pallipes* in the areas considered, we believe that additional cycles of *ex-situ* reproduction should be run in the Ponte San Giorgio – Camugnano (BO) incubator, in order to further refine the protocols developed during the project for target species reproduction in an artificial environment (in particular for the White-clawed Crayfish, given the modest numeric results obtained and the difficulties encountered during the breeding experiences conducted).

In fact, the material produced can be used to support the populations of *C. gobio* and *A. pallipes* within the perimeter of the Natura 2000 sites in the Tuscan-Emilian Apennine area, where the levels were lower despite the presence of the right environmental conditions for the species.

Moreover, the material can also be used to restock the populations in the case of catastrophic natural events.

Bearing in mind the description of adaptive management set out in the paragraph above, and based on our experience in this area, in order to encourage the reproduction and conservation of the two species it is more important to maintain the ecological efficiency of the rivers where the two target species were found than to

intervene with external contributions to the water courses. This goal can be pursued by creating small morpho-functional constructions made of lithic material found on the riverbed in the most suitable areas for reproduction and weaning, so as to create as many sites for refuge and reproduction as possible. This consideration is particularly relevant for the European bullhead.
Moreover, any tampering or intervention that could affect the integrity of these areas in those parts of the river where the protected species' habitats and populations are found must be banned, maintaining them for a distance of at least 20-30 metres from the outer margin of the riverbed when in full spate.
In this respect, it is worth remembering the fundamental ecological function performed by the plantlife growing along the banks of water courses, and which is essential for water-land interaction and the transfer of nutrients to the river from the surrounding land, through a dense hydrographical over- or under-ground network. The tree cover along the banks capture and decompose the nutrients (nitrogen and phosphorus), lower the water temperature (creating shady areas) and therefore increasing the oxygen levels, reducing the light penetration and thereby inhibiting the excessive development of aquatic plants which could in turn slow the water flow, as well as stabilising the banks, reducing soil erosion and the amount of sediment on the riverbed. Lastly, the banks encourage the settlement of a well-structured fish population in terms of both quality and quantity; moreover, it improves the habitat for amphibians, birds and small mammals.
The main ecological functions of the area surrounding the river can be summarised as follows:

- capture and decompose nutrients (nitrogen and phosphorus);
- lower the water temperature;
- increase oxygen levels;
- reduce light penetration, thereby inhibiting excessive growth of aquatic vegetation;
- stabilise the banks (reducing soil erosion and the amount of sediment on the riverbed);
- allow for the settlement of a well-structured fish community.

Another aspect to be adjusted is the fish seedings conducted within the area of the "Prato Apennine" SCI (Natura 2000 code: IT5150003).
The brown trout specimens that are usually introduced to Apennine water courses, despite some strong local attempts to produce and use native strains, are generally sourced from the Atlantic or are at the least hybrids.
The recent good practices published by AIIAD (Italian Association of Freshwater Ichthyologists) in the final report from the Salmonidae Work Group[97] set out a proposed classification system that takes the new concepts of ESUs (Evolutionary Significant Units) and MUs (Management Units) used in conservation biology into account. An ESU consists in one or more populations with partial genetic differentiation following significant evolutionary separation. An MU is any hypothetical population within a systematic group (distributed over a more or less extensive geographical area) which is sufficiently differentiated from the other populations as to justify separate management. The application of the new terminology to the *Salmo* family is partly justifiable given the genetic results currently available, and certainly pertinent on the basis of the different morpho-phenotypes described over the years.

The following classification is proposed for the Salmo family:

ESU/MU	COMMON NAME	DISTRIBUTION
Salmo trutta	non-native brown trout (mitochondrial haplotypes AT and DA)	Atlantic, Danube
Salmo marmoratus	marble trout (mitochondrial haplotype MA)	Po Valley (left bank of the River Po), Triveneto, Croatia and Slovenia
Salmo cettii	Mediterranean trout (mitochondrial haplotypes ME and AD)	Sardinia and Sicily, Lake Posta Fibreno, Lake Ninfa and some Tyrrhenian water courses (previous phenotype macrostigma)
Salmo ghigii	Mediterranean trout subspecies (mitochondrial haplotypes ME and AD)	Apennine, Adriatic and Tyrrhenian water basins
Salmo fibreni	Fibreno trout	Lake Posta Fibreno
Salmo carpio	Lake Garda carpione	Lake Garda
Salvelinus alpinus	Arctic char	Alpine and pre-Alpine lakes

According to this new classification, the seeding material used in the Apennine SCIs involved in the project and the nearby bodies of water should all be traced to the native Apennine strain, i.e. *Salmo ghigii*.
In order to perform this kind of selection, it is necessary to plan an experiment involving the selection of material to use for breeding, which must be certified by a scientific institution. For reproduction, incubator tanks can be used where possible on site: the incubator at Ponte San Giorgio (Camugnano – BO) or the one active at Casa al Rio (Cantagallo – PO) could be used and partly reconverted for this purpose.
The possibility of seeding should be planned in detail, limited only to the areas where the trout population is not dense enough and should aim only to support the trout population, it should be done exclusively using juvenile forms such as eggs, fry or fingerlings measuring 4-6 cm and with the medium-long term of making the fish populations self-sufficient, as in all Natura 2000 sites. Human intervention would therefore be limited only to re-population after catastrophic events, such as flooding or blights.

Good conservation practices for Italian crested newts and other amphibian species

In order to guarantee the conservation of *T. carnifex* and the other native amphibian species observed, along with their habitats, the availability and conditions of which are essential for their survival, we believe that monitoring of the 5 sites considered should continue even after the conclusion of the project, using the same monitoring protocol used during the project and the identification system for the individual specimens experimented by us. This continuation will allow us to pay careful attention to the demographic trends of the target species populations (in particular *T. carnifex*) and assess the results of the transfer operation performed in spring 2013, in order to understand both the factors of success and the potential or actual risks. These results and the consequent assessments are fundamental for deciding whether or not further fauna transfers should be performed.
The following briefly lists the good practices useful for the adaptive management of the habitats and species considered:

- Investigation of the ecological relations within the food chain by the target species, in particular between Anurians and Urodela (*Pelophylax bergeri/P. kl. hispanicus* and *Triturus carnifex*);
- Genetic profiling to define the local populations of the target species *T. carnifex*;

- Periodic control and maintenance of the wooden racks constructed around the hatchery pools for amphibians, in order to prevent them being accessed by invasive alien competitors;
- Numeric control and, where possible, local eradication of invasive alien competitor populations, using the methods tired during the project;
- Control of the proliferation of fast growing plant species such as *Phragmites australis* in all the areas considered, intervening by cutting where necessary;
- Allowing for plant coenosis to evolve and aquatic and hygrophilous plants to take hold, which are important for the life cycle of herpetofauna in general and *T. carnifex* in particular;
- Promote the work done in the population in general and schools in particular, to raise awareness among citizens of the issues covered by the project.

Based on the investigation and monitoring performed, the area of the Stagni di S. Ippolito meets the criteria for inclusion in the SCI/SPA/SIR "Ponds of the Florentine Plain" due to the presence of species and habitats of conservation interest.

Good conservation practices for the target bird species

The monitoring performed during the project allowed us to draw up specific guidelines for maintaining the wetland areas studied in a satisfactory state of conservation. There were three fundamental aspects to bear in mind from a management point of view: maintaining plantlife, controlling the water levels and controlling hunting activities.
Pruning plants is necessary to avoid the excessive proliferation of certain species, which could develop at the expense of others, leading to an excessive homogenisation and banalisation of the environment (reducing biological variety and diversity).
In the Bogaia wetland area, which is not subject to hunting activities, these interventions can be performed during the summer, from the second half of August onwards.
In the case of lakes subject to hunting activities, the plantlife can be controlled during the autumn, while in areas where aquatic birds stop-over or winter, ordinary lake maintenance should not extend beyond the first half of September.
In general, the diversification of the habitat created after the requalification measures should be maintained. In particular, the diversification of the bathymetry will need to be maintained, in order to guarantee the presence of shallow water alternated with deeper areas, encouraging different groups of species (e.g. shorebirds, ardeidae and diving ducks).
Regarding site 01 Lake Pantanelle, the trees in the central island should be encouraged to take hold, in order to improve the area's suitability for refuge or protection for species in the Ardeidae family.
Particular attention should be paid to mitigating the impact on birdlife caused by passing traffic (including heavy vehicles) along the highway currently being completed, right on the western side of the wetlands.
Moreover, it is essential to protect the vegetation that has sprung up in the uncultivated fields to the north of Lake Pantanelle, in order to encourage nesting, given that this area is highly frequented by birdlife, including the target species (Purple heron, Little Egret). This area (marshy grasslands), as already indicated for the Stagni di S. Ippolito area, has all the environmental features required for inclusion in the "Ponds of the Florentine and Prato Plain" SCI/SPA/SIR, extending the area and helping to maintain the conservation status in the populations of the target species living in and around the Pantanelle wetland area.
Lastly, the halophytic vegetation along the banks of sites 02 Lake Bogaia and 03 Lake Ombrone should also be

kept thick: in fact, the presence of “buffer zones” offers the bird species areas where they are less likely to be disturbed and helps create suitable refuge/nesting sites for many species.
The substantial drying out of site 03 Lake Ombrone, which was repeated annually each March throughout the monitoring period, is not admissible within an SCI/SPA, due to the clear negative impact on the life cycle of various target species. It is therefore necessary to ensure strict compliance with the instructions provided to the lake management (both in the Management Plan and in the current Hunting Regulations for the Province of Prato).
It is also important to succeed in limiting the proliferation of alien vegetation *(Reynoutria x bohemica*) growing along the banks of the River Ombrone, close by the lake, in order to avoid it spreading to the raised banks of the lake itself and replacing the native vegetation currently found there.
Lastly, for passeriformes such as *Lanius collurio*, the open spaces close to all three wetland areas need to be protected and in particular to the north of Lake Pantanelle, where uncultivated bushes and grassland is forming.
As these are areas where several interests coexist, it is fundamental to stress the importance of surveillance activities in the lakes in the SPA where hunting is permitted (Pantanelle and Ombrone), in order to guarantee compliance with general hunting regulations, the specific regulations governing all SPA in Tuscany and local regulations relating to the management of water levels and vegetation on the lake and surrounding areas, drawn up thanks to the knowledge accumulated during the “Water SCIs” LIFE project.

ABBREVIATIONS USED IN THE TEXT

AIIAD = Associazione Italiana Ittiologi di Acque Dolci (Italian Association of Freshwater Ichthyologists)
ANPIL = Area Naturale Protetta di Interesse Locale (Area of Local Natural Interest, as per Regional Law n. 49/1995)
BURT = Official Bulletin of the Tuscan Regional Authority
c. = call
CBD = Convention on Biological Diversity
DAISIE = Delivering Alien Invasive Species Inventories for Europe
DL = Decree-Law
DLgs = Legislative Decree
DCC = Municipal Council Resolution
DCP = Provincial Council Resolution
DCR = Regional Council Resolution
DGP = Provincial Government Resolution
DGR = Regional Government Resolution
DPGR = Regional Government Presidential Decree
DPR = Presidential Decree
ETS = European Threatened Species
ISPRA = Istituto Superiore per la Protezione e la Ricerca Ambientale (Higher Institute for Environmental Protection and Research)
IUCN = International Union for the Conservation of Nature
L. = Law
L.R.T. = Tuscany Regional Law
LRUNI = 2011 Red List of Breeding Birds in Italy
masl = metres above sea level
My = million years
obs. = sighting
ppm = parts per million
SCI = Proposed Site of Community Importance
s. = song
SHI = Societas Herpetologica Italica (Italian Herpetological Society)
SCI = Site of Community Importance, under the terms of the “Habitat” Directive (92/43/CE)
sni = site of national interest
SPA = Special Protection Area, under the terms of the “Birds” Directive (2009/147/EC)
SRI = Site of Regional Importance
sri = site of regional interest
SPEC = Species of European Concern
spp. = Multiple species (referring to a range of species from the same family)
TCI = Touring Club Italiano

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Leonardo Petri

Photo credits

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Fig. 2: Servizio Tecnico Bacino Reno – http://ambiente.regione.emilia-romagna.it/suolo-bacino/chi-siamo/servizi-tecnici-di-bacino/stb-reno

Figg. 3, 5: Marco Bagnoli, Daniela Quirino:

Fig. 4, 6: http://ec.europa.eu/environment/nature/natura2000/sites_hab/biogeog_regions/index_en.htm

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Figg. 83-84, 86: Lorenzo Lastrucci:

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